

THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME CI

BALTIMORE, MD.
1932

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THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 101

JUNE 1, 1932

No. 1

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

FORTY-FOURTH ANNUAL MEETING

Philadelphia, Pa., April 28, 29, 30, 1932

Redistributions of water measured in blood and tissues. E. F. ADOLPH,
M. J. GERBASI and M. J. LEPORE.

Translocations of water were studied in anesthetized dogs and cats by sampling blood, muscle, and sometimes skin, liver, and intestine at intervals of 2 to 12 minutes. All samples were analysed for water content, and some for chloride and total nitrogen. In heparinized blood samples specific gravity, hemoglobin, hematocrit, and plasma refractive index were also determined.

Whereas arterial blood samples were always representative, samples of any other kind of tissue were found to vary widely in composition, even when taken from the animal simultaneously. Thus, two pieces of the same muscle differed in percentage water contents with a mean deviation of ± 0.5 per cent of the mean value, and two muscles from the same leg differed by ± 1.5 per cent. Liver and intestine varied to the same degree; skin varied three times as much. For these reasons significant changes of water content could be identified only when the kind of tissue analysed differed by more than ± 3.0 per cent from the control tissue and the changes persisted for more than one set of samples. All variations in concentration were evaluated upon the assumption that the dry substance of the tissue remained constant.

The greatest modifications of water distribution were produced by intravenous infusions (20 to 55 cc. per kgm. of body weight) of various solutions of sodium chloride, acacia, gelatin, and blood.

During an infusion the mean arterial blood pressure usually rose, and in this period liver and often muscle showed increased water contents. Frequently the dilution of liver preceded that of muscle, each in turn recovering its normal concentration within a few minutes. The blood was diluted by the infusion, but if no colloidal material was contained in the infusion mixture the blood began to reconcentrate immediately after the infusion was completed. When the mixture contained gelatin or acacia the blood remained diluted and the tissues were little changed in water content. Transuded fluids often contained plasma proteins, and varied greatly and progressively in chloride concentrations.

Artificial hyperinsulism. FREDERICK M. ALLEN.

Experiments with administration of massive doses of insulin serve to clarify and correct prevailing conceptions of the action of insulin and the quantitative relations between insulin and carbohydrate. The antidoting of enormous doses of insulin does not require correspondingly enormous dosage of carbohydrate at one time and in fact cannot be accomplished by this means. Presumably there is a maximum rate of carbohydrate utilization for the organism, and sufficient glucose to supply this utilization protects against any excess of insulin for the time being, but the effects of massive doses continue longer than small doses and longer than can be antidoted by any quantity of carbohydrate which can feasibly be given at one time. Repeated administrations of carbohydrate are therefore necessary for protection against hypoglycemia. In suitably planned experiments an animal may thus be protected and then may be allowed to develop severe hypoglycemia several days after the insulin administration. The simplest explanation is that insulin can be stored in the body in larger quantities and for longer periods than heretofore supposed. The possibility of other explanations, the sites of the apparent storage, and the occurrence of insulin free or in masked form are among the questions to be answered by further experiments in progress.

The effects of feeding varied rations upon the hydrogen ion concentration of the intestinal contents of the domestic fowl. D. W. ASHCRAFT.

Experiments have been carried out to determine the effect of feeding milk on the hydrogen ion concentration at various levels of the intestinal tract of the domestic fowl. These experiments are the first step in a study of the effectiveness of milk products in the field control of coccidiosis.

The electrometric method using a type K potentiometer equipped with Clark's shaking electrodes was used. The contents of the duodenum, the ileum, the cecae and the large intestines were stripped into graduated tubes, diluted with three parts of re-distilled water, well shaken and centrifugated. The supernatant fluid was then tested. Preliminary tests with various dilutions showed that a dilution of one to four was the most convenient to use and gave the most consistent readings.

Six groups of birds of ten each were fed the following test-rations over a period of two weeks: a, 20 per cent lactose; b, 40 per cent dried skim milk; c, 20 per cent Kraco (dried whey); d, 40 per cent dried buttermilk; e, 20 per cent dried buttermilk; f, 20 per cent meat scrap. The last named ration, a milk-free product, was used as a control. The most pronounced effects in increasing the hydrogen ion content in the intestinal tract were produced in the ceca. The ability of these rations to increase the hydrogen ion concentration in the cecal contents was found to be in the order given above since the arithmetical means of the pH of the cecal contents were as follows: a, 5.10; b, 5.40; c, 5.95; d, 6.52; e, 6.74; f, 7.06.

Physical examination of the cecal contents of birds fed milk products always presented a characteristic yellow, cheesy, frothy mass, contrasted with the thick, non-gaseous, brownish, pultaceous mass normally found.

Humoral transmission of chorda tympani hormone. B. P. BABKIN, GEORGE W. STAVRAKY and ARMINE ALLEY.

If a small dose of physostigmine is introduced intravenously into an animal under anesthesia, stimulation of the peripheral end of the severed chorda tympani produces after a certain latent period a sharp fall of blood

pressure. This phenomenon was attributed to the liberation from the submaxillary gland during stimulation of some substance which has the effect of a parasympathetic drug and of which the destruction in the blood is prevented by physostigmine. In the investigation of one of the phases of this problem it was demonstrated that in a physostigminized cat with both submaxillary ducts cannulated and the chorda tympani and sympathetic nerves on both sides severed, stimulation of the chorda on one side produced a fall of blood pressure, and increased the secretion and accelerated the blood-flow in the opposite gland. Lowering of the blood pressure by arterial bleeding did not produce such an effect. Tying of the glandular vein on the stimulated side prevented this effect. Under analogous conditions the effect of the chorda tympani hormone could be demonstrated likewise in the case of the paralytic secretion of the submaxillary gland.

The effect of lumbar sympathectomy on the flow of blood in the femoral artery of the dog. EDWARD J. BALDES, J. F. HERRICK and HIRAM E. ESSEX.

Using the thermostromuhr method of Rein, a series of measurements has been made on the flow of blood in the femoral artery of the dog under the following conditions: First, the flow in the right and left femoral arteries was studied under local anesthesia. Although the flow compared favorably in each femoral artery, a variation was found in the absolute value in relation to the varying physiologic status. Second, the foregoing observations were repeated under general anesthesia. The flow of blood was found to be greatly increased. This increase may be more than 100 per cent. Finally, unilateral lumbar sympathectomy was performed on the dog. After full recovery from the effects of the operation, the measurements of the flow of blood were repeated. It was found that the increase in flow under general anesthesia was approximately equal to the increase following sympathectomy.

Cerebral localization of "hopping" and "placing" reactions in cats, rats and alligators. PHILIP BARD, CHANDLER M. BROOKS and THOMAS LOWRY.

In the case of the cat these postural reactions, first described by Rademaker (Das Stehen, 1931), are under the control of the region of the motor cortex (Bard, Arch. Neurol. and Psychiat., 1932, 27). The control is wholly contralateral. After unilateral decortication or unilateral removal of the sigmoid gyri the placing reactions (except the visual) are absent and the hopping reactions absent or feeble in the contralateral legs. These reactions are not modified by removal of temporal or occipital cortex or gyrus proneus. When one entire hemisphere and the motor region of the other are ablated the reactions are permanently and equally deficient in the legs of the two sides. They remain normal in the contralateral legs after extirpation of all cortex except the sigmoid gyri, gyrus proneus, and anterior third of the lateral gyrus, and this result is not modified by total ablation of the opposite hemisphere. This normal functioning of a cortical remnant shows conclusively that the cortical representation of these reactions is strictly localized and remarkably independent of all other cortical areas.

Repetition of the same operations has established the same general facts for the rat. In this lower mammal the reactions are under the control of a relatively larger fraction of the cortex, the anterior third. After effective cerebral ablation the hopping on adduction exhibits only slight

retardation, while the other hopping reactions are as deficient as in the case of the cat.

The alligator, an animal having a very primitive cortex, was found to possess one definite placing reaction and to show good hopping on adduction and retroflexion of the proximal joints. Removal of the entire brain cranial to the mesencephalon has no effect on these reactions. If, in addition, a lateral half of the midbrain be removed these reactions fail in the contralateral legs and after bilateral removal they are wholly absent. Ablation of the tectum has no effect on these reactions.

The excretion of iodine in experimental hyperthyroidism. B. O. BARNES.

The excretion of iodine in the urine and bile has been determined following the administration of desiccated thyroids, thyroglobulin, and thyroxine. Twelve normal dogs were fed desiccated thyroids (Armour's) for periods varying from one week to 92 days. The urinary iodine amounted to a little over 50 per cent of the total ingested. After the intravenous injection of thyroxine, the urinary iodine is considerably less. Iodine in the bile has been estimated in 10 dogs with biliary fistulas. Seven were fed thyroid in doses varying from 1 gram per kilo to a single dose of 100 grams. Feeding smaller doses for a week leads to only traces of iodine in the bile. With a single dose of 100 grams, a small amount of iodine is excreted through the gall bladder. On the contrary, small doses of thyroxine intravenously lead to immediate appearance of iodine in the bile.

Analysis of the cortical response to stimulation of the optic nerve. S. HOWARD BARTLEY.

From the cortex of the brain can be recorded action potentials, apparently resulting from spontaneous activity, which we have reason to believe are the action potentials primarily of cells rather than of fibers. The record led from any one point is complex, and involves superposition of the simpler rhythmic responses of several groups of cells. When an afferent nerve is stimulated, specific groups of potentials appear, superposed upon the record of the spontaneous activity. These potentials are obtained only from certain parts of the cortex, and differ for different nerves stimulated.

Following a single stimulus to the optic nerve of the rabbit, typically five successive potential elevations, timed at about $\frac{1}{5}$ -second intervals, appear in the record from certain parts of the optic cortex on the contralateral side. The question arises whether these five responses are repetitive responses of one group of cells, or successive responses of five groups of cells. Our evidence indicates the latter. First, rhythmic activity should be expected to follow a simple course, either to start with a maximal response and decrease progressively, or to rise to a maximum gradually and then die away. Of these five responses the amplitude may vary in many ways, and quite irregularly. A common sequence is a high first response, then two low responses, then one high one, and the fifth low; or the first may be low and a later one much higher than any other. Second, if successive rhythmic responses varied in form, they should vary progressively. Of these waves, a simple first response may be followed by complex waves, these by simpler ones again, etc. Third, a given position of the lead electrodes should record waves of the same potential sign for each repetitive response of a single element; of these five potentials recorded from a single position some may be mainly positive, some negative, and some diphasic. Fourth, rhythmically responding cells should show either regular intervals,

or regularly changing intervals between responses, and these responses do not exhibit such regularity. Fifth, if a second stimulus is applied to rhythmically responding cells before response to the first has subsided, the two effects should sum in some manner. Experimentally, if two effective stimuli are applied at less than 1-second intervals, the response to the second may begin before the response to the first has ended, and without altering the time relations of the later elements of the response to the first stimulus.

For these and other reasons of a similar character, we believe these records indicate the successive responses of separate groups of cells, probably of the different layers of cells of the cortex immediately below the electrode.

The rôle of water and chlorides in the production of edema in nephrectomized dogs. F. S. BARRY.

Ringer's solution injected subcutaneously in bilaterally nephrectomized dogs produces edema and prolongs life for an average of sixty hours.

A solution of 4 per cent glucose injected subcutaneously in bilaterally nephrectomized dogs neither prolongs life nor produces edema.

Mixtures of Ringer's and 4 per cent glucose solution sufficient to maintain the blood chlorides at a normal level do not produce edema consistently when injected subcutaneously into bilaterally nephrectomized dogs. They do however prolong life.

These results show that edema is not the factor concerned in prolonging the life of nephrectomized dogs and that "adequate" water plus chlorides must be administered to nephrectomized dogs to secure edema.

The determination in man of end systolic, lateral systolic, dicrotic and diastolic pressures by a modified Riva Rocci method. H. C. BAZETT.

Owing to the superiority of von Recklinghausen's criteria as evidenced by comparison of indirect measurements with direct measurements in dogs, an oscillatory system has been developed which appears to give truer indications of the wave characteristics in the compressed vessel. The system employs 3 bags one within the other. A large outer bag (12 cm. diameter) compresses the limb, as well as the two inner bags, which lie between it and the limb; a small innermost bag is connected to a segment capsule and records the pulsations of the vessel; between these two bags lies an intermediate bag of moderate size which protects the innermost bag from pulsations transmitted to the larger compressing bag from the vessels above the cuff. It is connected to an air tight box which encloses the segment capsule and so maintains similar pressures on the two sides of the membrane. Definite criteria for end systolic and diastolic pressures are obtained. In addition the lateral systolic pressure and the pressure of the dicrotic wave can usually be determined. Comparisons of lateral pulse pressure with cardiac output are being made.

Further experiments on chronic decerebrate cats. H. C. BAZETT.

The author with W. G. Penfield¹ has described technical methods for production of chronic decerebrate cats but success was rarely attained. The method has now been modified; the animals are kept in a moist warm room (dry bulb 26°C., wet 22°), they lie on copper tables heated by

¹ Bazett and Penfield. *Brain*, 1922, xlv, 185.

lamps which are regulated by a control system operated on an individual basis according to the animal's own rectal temperatures. This is achieved by the use of electrical resistance thermometers connected to a Leeds and Northrup recorder through a selective switch. A series of animals (up to 6) can be so regulated; each animal is connected to the galvanometer system for $1\frac{1}{4}$ minute every 10 minutes, and the heating lamp is turned on or off through a mercury contact relay and is so maintained until the next period of sampling. Cats may be kept for periods up to one month, and 50 per cent of the animals can be preserved for weeks provided they survive the immediate effects of the operation.

The presence of hypothalamus above the pituitary stalk has proved essential for life beyond one week. This tissue is effective in preserving life, even when it has no nervous connection with the rest of the nervous system. Its value must depend on some chemical factors. In its absence polyuria is a prominent symptom.

Reflexes are usually brisk and more complex than in the acute animal, but no capacity to prevent a fall of body temperature or to shiver on exposure to cold has been demonstrable in spite of the assignment by some workers of such responses to centers in the medulla.

Alterations in blood lactic acid as a result of exposure to high pressures of oxygen. JOHN W. BEAN and JOHN HALDI.

The experimental animals used in this study were dogs weighing from 8 to 12 kilos anesthetized with morphine and urethane. The apparatus recorded ventilation, blood pressure and pulse rate on smoked paper. The oxygen was obtained from commercial supply tanks (Linde Co.). Lactic acid determinations were made by the Friedemann, Cotonio and Shaffer method. Blood samples were taken from the carotid blood circulating through an external glass tube provided with a sampling stopcock. An intravenous injection of saline equal in amount to the blood drawn was made after each sample.

The general procedure was to expose the animal to oxygen at one atmosphere for about thirty minutes, rapidly raise the pressure to five atmospheres, continue the exposure at this pressure for thirty minutes, then decompress in stages over a period of such duration as to avoid the possibility of effervescence which might result from too rapid decompression. Decompression was followed by a prolonged exposure to one atmosphere of oxygen. Blood samples were taken before, during and after exposure to five atmospheres of oxygen.

The usual findings with respect to blood lactic acid were a, a slight decrease or no change during exposure to one atmosphere of oxygen; b, a slight increase in a few experiments and a marked increase in the majority of cases during exposure to high pressure; c, a decrease during and after decompression and a return toward the precompression lactic acid level. The deleterious and irreversible effects of oxygen at high pressure were no doubt a factor in preventing recovery in several of the experiments. Without exception the respiratory minute volume was increased during the period of high pressure.

Changes in volume flow of blood resulting from chemical stimulation of the carotid sinus. THEODORE G. BERNTHAL.

The influences of certain types of chemical stimulation of the carotid

sinus upon blood volume flow in the femoral artery of the dog were determined.

All branches of each common carotid artery except the internal carotid were ligated. One carotid sinus nerve was destroyed while the other was left intact. NaCN , Na_2S , NaHCO_3 , and Na_2CO_3 were injected into the common carotid arteries. Volume flow of blood was measured in the right femoral artery using the thermo-electric method of Gesell and Bronk. Mean blood pressure was artificially maintained at a constant level.

Injection of NaCN into the intact carotid sinus was followed promptly by a short period of slightly decreased femoral flow succeeded by a marked increase of flow of longer duration. Return to the pre-injection level followed. Often there was a secondary wave of increased flow. NaCN injected into the carotid artery on the side of the denervated sinus usually produced no change in femoral flow, although a moderate late increase in flow could often be elicited if doses of sufficient concentration were used.

The effects of Na_2S injections were practically identical with those of cyanide.

Injections of NaHCO_3 into the carotid on the side of the intact sinus produced an initial small decrease followed by a marked increase in femoral flow. Injections into the carotid on the side of the denervated sinus sometimes produced no effect, but usually resulted in an increased femoral flow. The response on the denervated side was always later in appearance and always definitely smaller than that on the intact side.

The effects of injection of Na_2CO_3 were an initial decrease followed by a very sharp, extensive and prolonged increase in femoral blood flow. There were no differences in response to injections of Na_2CO_3 on the denervated and on the intact side.

The control of the deposition of liver fat. C. H. BEST, J. M. HERSHEY and M. E. HUNTSMAN.

Experimental results previously reported from this laboratory showed that a condition, characterized by fatty degeneration of the liver, which occurs in diabetic animals kept on a lean meat and sugar diet could be alleviated by the administration of "lecithin." These findings have been repeatedly confirmed. It has been observed that the onset of the characteristic condition can be accelerated by the addition of fairly saturated fats to the diet. The "lecithin" effect is not due to the presence of vitamins A, B, C or D.

More recently it has been shown that the deposition of large amounts of fat in the livers of normal white rats produced by a diet high in saturated fats can be completely prevented, or reduced in amount, by the oral administration of large or moderate amounts of crude or purified lecithin from egg yolk or beef liver. No evidence has been obtained that the feeding of the necessary amount of lecithin causes any increase in fat excretion. When amounts of glycerophosphate, sodium oleate, amino-ethyl alcohol and choline, which might be derived from an effective dose of the lecithin preparation, have been administered, negative results have been secured in all except the choline-fed animals. In these, the deposition of liver fat has been consistently prevented whenever adequate amounts of choline have been provided. Diets containing naturally occurring lecithin inhibit the deposition of liver fat.

The formation of free HCl from NaCl in the stomach and in vitro. R. BEUTNER and M. CAPLAN.

According to Apperly¹ the formation of free HCl in the stomach runs parallel to the bicarbonate content of the blood. It seems, therefore, that NaCl is split in the glands of the stomach into HCl and NaOH, the latter being immediately transformed into NaHCO₃ by the CO₂ present. In the place of NaOH a sodium salt of a very weak acid might be formed intermediately, which would likewise be transformed into NaHCO₃. No explanation for this gastric acid formation is afforded by any of the well-known processes which may split a salt into free acid and base.² *We have found an in vitro reaction by which NaCl is split directly into HCl and a soap, like sodium oleate, or another salt of a weak organic acid.* Such a reaction, which is contrary to that occurring usually in aqueous solution, actually does occur if we use the weak organic acid, dissolved in a suitable water immiscible solvent, and agitate this solution with a NaCl solution. As a solvent we may use, e.g., cresol or guaiacol. In this, a small amount of oleic acid is dissolved. When such a solution is shaken with NaCl solution, HCl is formed, as can be demonstrated by titration. Or, a solution of oleic acid in olive oil may be used. This was done, a long time ago by Clowes,³ who was the first to observe this kind of a reaction. In coöperation with Doctor Clowes,⁴ we have also examined a solid substance, nitrocellulose, which also forms HCl from NaCl.

In general, a formation of HCl from NaCl is observed with all these mixtures or substances which—when inserted between two solutions of different concentration—give rise to a comparatively large electromotive force, viz., the so-called maximal effect of concentration, as is to be expected according to a theory developed by one of us.⁵

Control experiments were necessary since most of the water immiscible substances have also a slight water solubility, and hence give off traces of acid even if shaken with pure water. However, if they are shaken with NaCl solutions, very much more acid is formed. Further controls exclude the possibility of a "salt error."

Our assumption is that the type of reaction studied is one possible factor for gastric acidity, but there must be additional factors which remain to be discovered.

Certain time-relations of the visual pathway. G. H. BISHOP.

On direct stimulation of the optic nerve of the rabbit by single shocks, the first cortical response (action potential wave) appears after an interval of from 4 or 5 up to 25 sigma, depending on the strength of stimulus and upon the momentary state of the cortex, which varies rhythmically. When this response is large, either because many fibers are stimulated or because the cortical pathway is in a non-refractory phase of its spontaneously rhythmic activity, the time between stimulus and response is short, and vice versa. The time to maximum of this potential is 5 sigma, its total duration 15 or more sigma. This first electrical response is followed by several others, all resulting from the one stimulus. The time interval between the first and second responses is of the order of 150 sigma, and

¹ Journ. Physiol., 1931, lxxiii, 331.

² This point and other hypotheses relating to it will be discussed extensively.

³ Journ. Physical Chem., 1916.

⁴ Unpublished findings.

⁵ See R. Beutner, Entstehung elektrischer Stroeme in Geweben.

varies only slightly if at all in time, either with the strength of the nerve stimulus or with the height of the first response. We have been interested to know how the impulse is spending its time between the responses of the various elements of its pathway.

A direct lead from the optic tract beyond the chiasm but just before the tract reaches the thalamus, gives a record of the optic nerve action potential. The time for conduction of the fastest fibers is of the order of 1 sigma, and does not vary with the number of fibers stimulated. Assuming one synapse in the thalamus and one in the cortex to be passed before the first cortical response, and one sigma conduction time in the optic pathway from thalamus to cortex, there is left a maximum of over 20 sigma and a minimum of 2 or 3 sigma to be divided between these two synapses. These values of 1 to 10 sigma per synapse are not unreasonable as compared to synapses in the spinal-cord reflexes (one sigma or more) or cervical sympathetic ganglion synapses (15 to 20 sigma). Assuming that the axon of a cell responds at about the time of the maximum of the activity of that cell, and that these successive cortical responses are due to different groups of cells close to the lead electrode, the conduction time between the cells giving the first and second responses here can be ignored, and the second group of cells must respond only after upwards of 100 sigma from the time the exciting impulse reaches it or its dendrites.

The variation in time of the first response, after stimulation of varying numbers of fibers, must mean that the impulse of one fiber affects several cells, and the impulses of several fibers affect each cell; and that the greater the number of impulses received by the cell, the sooner it responds. The second group of cells responding does not seem to behave in this way, the time of its response after the first cell response varying little with the amplitude of the first response.

Stimulated once per second with a submaximal shock the first response varies in height. An interval can be found, however, usually about $\frac{1}{3}$ second, that gives a constant response to a submaximal stimulus. This is taken to indicate that the pathways stimulated are spontaneously and cyclically active, and the cycle is 3 per second, the cortex being driven as if by a pacemaker. Now if the stimulus is increased to maximal, a second shock following a first by one cycle ($\frac{1}{3}$ second) is no longer effective, but is maximally effective if it follows by $1\frac{1}{2}$ cycles. This might mean that the pacemaker is now being driven, and that at the end of one cycle after its maximal response the pathways are all refractory, but all recover $\frac{1}{2}$ cycle later.

The findings suggest that the effects of peripheral stimuli reaching the cortex involve not only the specific activity of its cells induced by the afferent impulse, but the alteration of the spontaneous activity already going on in the cortex. It is obvious that our conditions of stimulation do not duplicate the effects of light stimuli falling on the retina. The latter process presumably results in repetitive stimulation of the optic nerve fibers, not all in the same phase, and therefore more difficult to analyze.

Effects of polarization of nerve fibers by extrinsic action potentials. E. A. BLAIR and JOSEPH ERLANGER.

For the interpretation of certain experimental data it seemed desirable to ascertain to what extent the reactivity of inactive fibers of a nerve is affected by the potentials developing in active fibers. For the observa-

tion and recording of the action potentials in these experiments the cathode ray oscillograph of Von Ardenne replaces that of Johnson in our plant. The pictures on its screen are so brilliant that single deflections at the highest necessary speeds can be sharply photographed. Its lower voltage sensitivity is easily compensated by higher amplification.

Two action potential volleys started one slightly behind the other in groups of similar fibers travel long distances without changing their relations to each other. It follows that such current as leaks from one of the fiber groups into the fibers of the other is without effect on their inherent propagation rates.

The current determined by a maximum monophasic action potential when led from one nerve into uninjured parts of another is without demonstrable effect on the irritability of the latter, even when its irritability level is raised just to the firing off point by local cathodal polarization. But when, under these circumstances, the action potential is introduced through leads resting one on injured end, the other on intact side, a slight additional stimulation of fibers occurs.

An action potential volley traveling through one group of fibers composing a nerve is without demonstrable effect upon the irritability of a comparable but inactive group. Even when the irritability of the latter is raised locally by a polarizing current to a level just short of response, the fibers are not stimulated by an action potential traveling through the former, provided the terminals of the polarizing current rest on uninjured nerve.

The relation of insulin to liver glycogen. Part II. R. C. BODO, F. CO TUI and L. A. FARBER.

In 48 hours after pancreatectomy in dogs, the liver is practically glycogen free, if no food has been taken during this period. If at the end of 48 hours, a continued infusion of sugar and insulin is made, the animal being anesthetized with morphine and chloralose, there is a gradual storage of liver glycogen, amounting to as much as 1 per cent in 5 hours, and 3 to 4 per cent in 7 hours. If sugar without insulin is infused, there is also a glycogen storage but the amount is not more than 1 per cent in 7 hours. If no anesthetic is used, and sugar is given by hourly subcutaneous injections, at the end of 5 hours the liver contains about 1 per cent of glycogen. If after the pancreatectomy, sugar is given two or more times a day by subcutaneous injection, at the end of 48 hours the liver contains about 1 per cent of glycogen. If at the time the animal is anesthetized and a continued infusion of sugar is made for 7 hours, there is no significant further rise of glycogen, but if insulin is added to the sugar infusion, the liver glycogen rises to 3 or 4 per cent in 7 hours.

From these experimental results it is seen that the diabetic animal is able to store liver glycogen 48 hours after pancreatectomy if sugar alone is given. If insulin is given with the sugar, the storage is greater. Possible explanations of these findings are discussed.

The effect of carbamates on the electric potential of frog skin. EDGAR JOHN BOELL and A. B. TAYLOR.

The effects of different concentrations of methyl, ethyl, n-propyl, n-butyl, n-amyl, and phenyl carbamate in Ringer's solution on the electric potential of frog skin have been quantitatively and comparatively studied.

The skin of the frog was divided into eleven pieces which were fastened over the mouths of tubes by means of rubber bands, and were bathed in Ringer's solution maintained at a temperature of $25^{\circ} \pm 0.5^{\circ}\text{C}$. Determinations of E.M.F. were made potentiometrically. After a preliminary period, of approximately two hours, in Ringer's solution, the skins were transferred to one containing a certain percentage of carbamate and were finally again placed in Ringer's.

Inasmuch as the magnitude of the electric potential of different pieces of skin, from the same or from different frogs, varied widely, but since the electromotive behaviour of different skins was qualitatively similar, the E.M.F. in millivolts was transposed to per cent in order to permit of quantitative evaluation of the effect of carbamate. The potential at the time of application of carbamate was arbitrarily designated as 100 per cent. In terms of per cent the skins from the same or from different frogs behaved quantitatively alike.

The normal E.M.F. of frog skin gradually decreases with time. The application of carbamate to skin causes a reversible diminution of E.M.F. which is a function of the duration of application and of concentration. Methyl carbamate, in concentrations sufficient to cause appreciable reduction in potential, injures the skin so that complete recovery does not occur, or occurs only very slowly. In general, however, the higher members of the homologous series are more effective than the lower members. The magnitude of the E.M.F. after restoration of the skins to Ringer's may be greater than, equal to, or less than the potential at the time of addition of carbamate.

Some observations on glycemia in turtles. EPHRAIM B. BOLDYREFF and JEAN F. STEWART.

This preliminary report is the result of blood sugar determinations made in a group of turtles of the following species: *Chrysemys marginata*, *Emys blandingii*, *Chelydra serpentina*, *Aromochelys odoratus* and *Gratemys geographica*.

The number of animals used was 60. Blood sugar estimations were made by the Folin-Wu method, the blood being obtained by decapitation for single determinations; and by intracardiac puncture under anesthesia when several observations were made. The normal range of blood sugar content was found to be between 0.035 to 0.070 per cent. Intraperitoneal administration of Nembutal or 40 per cent alcoholic solution of chloretone seemed to have some hyperglycemic effect; constant blood sugar levels could be maintained, however, in anesthetized animals by proper adjustment of dosage. Hibernation causes a marked variation in the amount of blood sugar in the turtle (*Chrysemys marginata*); in the early stages of winter sleep a pronounced hyperglycemia is present, the intensity of which seems to decrease in the course of time. Disease of the pancreas is another condition associated with high blood sugar values. Pancreatic cysts in one of the species (*Chrysemys marginata*) is a not infrequent affliction caused by parasites and foreign matter coming from the intestine, resulting in necrosis of the adjacent tissue with infection and inflammation of the pancreatic ducts. Similar pancreatic disease was also found in another species, *Chrysemys elegans*, of the same genus.

The administration of large doses of insulin demonstrated the remarkable resistance of turtles to the hypoglycemic property of this drug. Following

its injection an increase in blood sugar was occasionally observed in anesthetized animals. It was found nevertheless that a diminution of blood sugar could be obtained by the use of insulin. Hypoglycemic effect was observed with dosages varying from 6.6 to 40 units. In one instance (*Chelydra serpentina*, weighing 2600 gm.) the amount of blood sugar was markedly decreased following the intracardiac injection of 24 units of insulin. For 3 days prior to this the turtle had been kept on a fasting condition and at a temperature of 31 to 32°C. The reduction in this case was almost 50 per cent of the initial fasting level and attained the low value of 15 mgm. per 100 cc. of blood. Another example of a marked effect of this hypoglycemic drug was observed in *Emys blandingii*, weighing 1500 grams; a series of subcutaneous injections of insulin were made during a period of 15 days, the total dose amounting to 201 units. Following the injection of 40 units the symptoms first appeared in about 36 hours (single injection of 40 units); the animal becoming very sluggish with an increased respiratory rate. Finally it went into hypoglycemic coma with blood sugar reduced to 0.007 per cent, the blood becoming non-coagulable.

Both commercial and crystalline insulin were employed with practically identical results.

Lactic acid utilization following complete removal of the liver. JESSE L. BOLLMAN and FRANK C. MANN.

Removal of the liver is followed by an increase of lactic acid in the blood and urine. A considerable amount of the increased lactic acid may be dependent on ether anesthesia and operation since the values approach normal with recovery from the anesthetic. Hypoglycemia does not increase the production of lactic acid except when it is accompanied by muscular twitchings. Administration of glucose has no specific effect on the lactic acid of the blood except to prevent symptoms of hypoglycemia. An increase of lactic acid in the blood frequently develops in the terminal stages after hepatectomy.

In the absence of the liver large amounts of lactic acid appear to be utilized. Following the intravenous injection of lactic acid only a small portion is excreted in the urine and the amount in the blood returns to its level before injection. Since lactic acid is not a substitute for glucose in the prevention of symptoms of hypoglycemia of hepatectomy, we were unable to prove definitely that glycogen formed in the muscles from lactic acid without the administration of glucose. Epinephrine does not markedly increase the amount of lactic acid in the blood or urine following hepatectomy, and glycogen may be formed in the muscles from glucose in the presence of large amounts of epinephrine which inhibits the formation of glycogen in the muscles of normal animals under similar conditions.

Return of indirect excitability in curarized preparations, following tetanization of the motor nerve. T. E. BOYD.

It has been reported previously that the action of physostigmine, in removing curare paralysis in the cat, is facilitated if the motor nerve has been tetanized for a time before physostigmine is administered.

Simple tetanization of the hypoglossal nerve will restore indirect excitability of the tongue muscle in lightly curarized cats. The effect may be transient or prolonged, depending on the depth of curarization and the duration of the period of tetanizing. The recovery of conduction from

nerve to muscle is not due to vascular changes in the tongue, nor to any influence of sympathetic fibers in the hypoglossal. It is suggested that previous activity, in the nerve, may so modify the "nerve impulse" that it becomes more effective in passing a curare block.

We have not been able to demonstrate a similar behavior in frog preparations.

The analysis of the initial heat in smooth muscle. E. BOZLER.

The rate of initial heat production in the different phases of the isometric contraction of the retractor of the pharynx of the snail is analyzed by means of the method of Hartree and Hill. A very quickly recording and sensitive arrangement has been developed which allows an analysis in intervals of 0.1 second. There are three maxima in the rate of heat production; the first shortly after the beginning of the contraction, the second at the maximum of tension and the third which coincides with the turning point in the relaxation curve. The rate of heat production during the relaxation diminishes with the square of the tension. The total amount of relaxation heat is proportional to the square of the maximum tension. A quantitative explanation of these relations and of the absolute amount of relaxation heat can be given if the relaxation heat is regarded as the energy which derives from the potential elastic energy of the muscle.

Seasonal differences in the survival of cats after adrenal removal. S. W. BRITTON.

Opportunity to make observations on the survival of a large number of adrenalectomized cats has been presented in this laboratory during the past three years. In about 400 experiments, analysis of the survival periods during different seasons of the year was possible in over 250 cases. Because of the various terminal procedures employed, the time of appearance of symptoms in operated animals was taken as the standard criterion, rather than the actual time of death. After symptoms of insufficiency had appeared, it was noted that death almost invariably resulted in 24 hours. There were found to be consistently longer periods of survival throughout the colder months of the year—November, December, January and February—than at any other time. The shortest life-spans after adrenal excision were observed in June, July, August and September. Previous experiments showing that summer-operated marmots die within a few days while fall-operated animals live throughout the winter, and die with the usual symptoms of adrenal insufficiency in the spring, appear to be related to the present findings.

Effects produced by cortico-adrenal extract on normal animals. S. W. BRITTON and H. SILVETTE.

Young rats have been found to be particularly responsive to cortico-adrenal extract, and they may be used for biological assay of the material. Within two or three hours after the administration of extract the muscle glycogen may be augmented 100 per cent, and the liver glycogen 100 to 200 per cent, above the values observed in normal controls. There are also associated elevations in the blood sugar levels from 50 to 100 per cent above those observed in the control cases. The glycogen and glucose values remain at abnormally high levels for at least four or five hours after the initial increases occur. In all cases the changes which were observed

in the young animals used (rats weighing between 40 and 60 grams) were sharply and consistently defined. There were no significant changes in the blood lactates and the serum calcium and phosphorus levels.

On the apparent principal function of the adrenal glands. S. W. BRITTON.

The effects of adrenalectomy on carbohydrate metabolism have been observed to be profound in character, and are primarily responsible for eventual death of the animal. Blood glucose and liver glycogen are chiefly affected; these show marked reductions from the normal levels. There were also found consistent decreases in muscle glycogen, and increases in blood lactates. The glycogen of heart muscle was not reduced. In pancreatectomy and in hepatectomy carbohydrate metabolism was affected in no more critical degree than in cases of experimental adrenal insufficiency. The glycogenic and glyceemic changes which occurred after complete adrenalectomy were not observed in cases of extirpation of the adrenal medulla alone.

Administration of cortico-adrenal extract (Swingle-Pfiffner) brought about recovery of animals which were in the terminal stages of insufficiency apparently through initial restoration of normal carbohydrate values. Increments in circulating sugar to the normal or even to hyperglycemic levels, which occurred early after extract injection and were invariably associated with the disappearance of insufficiency symptoms, appeared to be of primary importance in effecting recovery. Restoration was completed with the subsequent reestablishment of hepatic and muscle glycogen values, and the diminution of blood lactic acid.

Emphasis is placed on the extreme fluctuations in blood sugar and liver glycogen which are observed during the development of and recovery from adrenal insufficiency. Cortico-adrenal extract increased the blood sugar and the liver and muscle glycogen values in normal animals. None of the results were produced by equivalent amounts of adrenalin similar in concentration to that found in cortical extract (1:2,000,000).

Completely adrenalectomized animals showed a markedly reduced ability to store liver glycogen. Normal individuals which were injected with glucose stored from eight to ten times as much glycogen as operated animals.

The evidence indicates that the cortico-adrenal tissues (and their pertinent hormone as contained in our extracts) are primarily concerned in the control of carbohydrate metabolism in the body. Since the cortex represents that part of the organs which is essential to life, the conclusion is derived that the regulation of carbohydrate metabolism may be considered the principal function of the adrenal glands.

The response of end organs in the carotid sinus. D. W. BRONK and G. STELLA.

We have recently described the nature of the nervous discharge from the carotid sinus and its relation to the arterial pulse curve and the mean blood pressure. In order to analyze the mechanism of the receptors in the walls of the sinus under more easily controlled conditions, the sinus has been isolated and perfused and afferent impulses from a single end organ photographically recorded. The sense organ responds to a constant pressure with a discharge of a regular sequence of impulses at a frequency which is a function of the pressure up to about 200 mm.Hg, beyond which there is little further increase in frequency. The maximum change in frequency

is in the pressure range to be found in the intact animal. At successively higher pressures more receptors are found to be discharging in preparations in which several end organs remain in connection with the nerve, thus illustrating the fact that intensity of stimulus generally results in an increased number of afferent impulses as a result of an increased number of end organs responding as well as an increased frequency.

Observations on the response during the development of increased endo-sinusal pressure and several minutes thereafter show that this type of end organ adapts slowly, the discharge continuing indefinitely at an only slightly decreased frequency. If the pressure be decreased from one level to another the impulses stop and after a variable period begin again, gradually speeding up to a frequency characteristic of the lower pressure. No satisfactory explanation of this phenomenon is thus far available.

The activity of the end organs is dependent upon the composition of the arterial blood. Because of the possibility of controlling the endo-sinusal pressure and its rate of development these studies on the isolated sinus have made it possible to determine the influence of chemical factors on its nervous response, undisguised by accompanying variations in the form of the pulse curve and mean blood pressure.

A contracture phenomenon in cross-striated muscle. DUGALD E. S. BROWN and DAYTON J. EDWARDS.

When a cross-striated muscle, immersed in blood serum, is subjected to an hydrostatic pressure above 2000 pounds per square inch a slowly developing and prolonged contraction results. In the retractor penis muscle of the turtle at 4°C. the "contracture" begins immediately after the application of the pressure. The tension increases to a maximum and then decreases at a slow rate so that a tension slightly less than the maximum is maintained for a long period (over 5 minutes). If the pressure is maintained the tension gradually falls and approaches the base line. A sudden decrease in pressure at any time during the contracture results in an abrupt increase in tension to a maximum and a subsequent relaxation. As the contracture can be obtained repeatedly on the same preparation it should be classed definitely as a *reversible* contracture. The development of tension to the maximum as a function of a time in the contracture is described

by the equation $K = \frac{1}{T} \ln \frac{a}{a-x}$ in which a is the total tension at the maximum, x the tension at the time T , and K the velocity constant. The total tension a varies directly with the pressure. It increases as a function in pressure along an S-shaped curve and is approaching an upper limit at 8000 pounds pressure. At this pressure level the total tension may be as great as 90 per cent of the total tension in a maximal tetanus at the same pressure. The largest values for the total tension at any pressure are obtained from muscles immersed in blood serum of the turtle. An imperfectly prepared Ringer's solution has the effect of diminishing the total tension to be obtained from the muscle at all pressure levels. Repeated applications of pressure, especially at low temperatures, reduces the tension to 10 per cent or less of the initial contraction at the same pressure, but a depressed preparation of this sort, if returned to a temperature of 20° for a period of 2 hours, will again give tension values comparable to the original.

The velocity constant K is independent of the pressure over the range

of 3000 to 6000 lbs. in preparations in which the contracture is large. On the other hand, the value of K tends to decrease with an increase in pressure at temperatures from 0 to 5° where the contracture is small (a high pressure threshold). The data at hand do not admit of establishing with certainty a quantitative relation between the total tension and the action of pressure on the velocity constant. The velocity constant K is determined by the temperature and has a temperature coefficient $Q^{10} = 6$, (0 to 10°C.).

Ebbecke has made observations on pressure contracture and he advances the view that this phenomenon does not involve the excitable system of the cell. This conclusion implies, therefore, that the contracture provoked by pressure results from some direct action of the compression on the contractile mechanism per se. Our results are in agreement with this view and they provide evidence also that pressure modifies the chemical as well as the physical (visco-elastic) factors of the contractile mechanism.

Is levulose converted to dextrose by the intestinal mucosa? G. E. BURGET, PHILIP MOORE and ROBERT LLOYD.

Solutions of levulose were introduced into chronic closed loops of ileum in dogs and into isolated living segments of rabbits' small intestine kept in oxygenated Ringer's solution at 37°C. No glucose was found in the loop fluid after exposure to the mucosa for one hour. The Ringer's solution in which the living segment containing levulose was suspended for one hour showed levulose but no glucose.

Under anesthesia laparotomy was performed upon six dogs and blood taken from the mesenteric vein from the closed loop while absorbing levulose. In all experiments levulose was found in the mesenteric blood while samples of heart's blood taken at the same time showed none.

The evidence obtained from these experiments indicates that levulose is normally taken up by the intestinal tract as such. However, this work does not exclude the possibility that in the absence of the liver the intestinal mucosa may convert levulose to dextrose.

The distribution of sugar between corpuscles and plasma. DEA BAILEY CALVIN.

Previous investigators have studied the distribution of sugar and other constituents between corpuscles and plasma, and have reached divergent conclusions. Somogyi is of the opinion that the corpuscle:plasma ratio of blood sugar is about 77:100. Folin and Svedberg consider the ratio to be 60:100. It seemed to the author that a study of distribution on the basis of corpuscle and plasma water would be of interest.

The Somogyi Copper-Sodium Tungstate method was used for the preparation of filtrates. Sugar was determined by the Shaffer-Hartmann method. Total solids were determined by drying weighed amounts of whole blood and plasma to constant weight at 102°C.

The data indicate that the ratio of corpuscle to plasma sugar is below 0.70, if corpuscle and plasma *volume* are used as a basis of calculation. If the corpuscle and plasma *water* are used as a basis for comparison, however, the ratio approaches unity. It seems, therefore, that the distribution of sugar between corpuscles and plasma is determined to a large extent by diffusion in the available water present.

Further work is in progress in an effort to determine the effect of *free*

and bound water on this ratio. Other diffusible blood constituents are being investigated by this same procedure.

A convenient pneumograph. EMMETT B. CARMICHAEL and LOUIS C. POSEY.

This pneumograph may be assembled in almost any physiological laboratory and can be used to record the respiratory frequency of laboratory animals without sacrifice of the latter. The set-up is convenient for measuring the respiratory rate of guinea pigs, rabbits, rats, mice (adults), dogs, and cats. A rate as high as 200 per minute may be easily recorded.

The equipment required for the pneumograph is as follows: one muscle-lever, one extension rod with axis, one small and one large weight, one ring-stand, one right-angle clamp, one kymograph, towels or waste, a metal plate (a kymograph fan will do), a piece of string and one animal board.

The arrangement of the animal, as seen in figure 1, makes it possible to take advantage of the fact that the thoracic and abdominal walls expand and contract during respiration, and thus to control the direction of the motion. The animal is placed on the animal-board with the ventral side up and is packed by means of towels or waste so that the major part of the

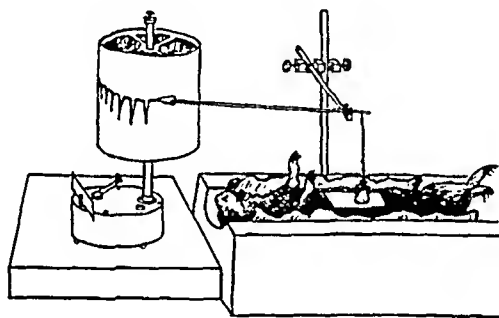


Fig. 1

motion is vertical. When anesthetics are used, it is well to place a towel over the animal's trunk to prevent loss of body heat.

When the animal is in position, the larger weight is allowed to rest upon the metal plate, which has previously been placed on either the abdomen or the thorax, depending upon the desired amplitude. This weight is attached to the muscle-lever by means of a string. On the opposite end of the lever (the writing end), may be suspended a smaller weight to increase the sensitivity. On inspiration the lever makes a down-stroke and on expiration it makes an up-stroke.

The principle of the lever as explained herein can be used for measuring the rate of respiration of human beings if one will shorten the lever and elevate the kymograph.

Magnesium secreted in the sweat and its relation to the calcium content.

RALPH CARPENTER and G. A. TALBERT.

By the method of McCrudden, magnesium was found in the sweat in 76 out of 79 determinations on six different subjects. There was an average of 1.19 mgm. per 100 cc. There was, however, a maximum of 4.5 mgm. found in two instances.

In 103 determinations for calcium we obtained an average of 4.68 mgm. per 100 cc. While these two elements bear on an average a ratio of 4 to 1, yet in many instances this ratio was not by any means adhered to. In fact, we found in three cases that the magnesium was secreted in excess of the calcium.

The recovery heat-production of mammalian muscle. McKEEN CATTELL and EPHRAIM SHORR.

It has been shown by Richardson, Shorr, and Loebel that thin strips of dog muscle maintain normal oxidations for a long period after removal from the animal¹ and that under such conditions the glycogen-lactic acid cycle may be demonstrated.² These facts have suggested the use of mammalian muscle for a study of the recovery heat production following activity. For this purpose we have used thin strips of the scalenus muscle in connection with the myothermic technic of A. V. Hill. The total heat liberated in response to a series of 5 single shocks and to a one second tetanus was determined by the area of the galvanometer deflection-time curve. After killing the muscle, deflection time curves were obtained for periods of control heating similarly distributed in time. The difference in area between the live and control curves, after reducing to the same maximum, gives the recovery heat. All observations were made in an atmosphere of oxygen at a temperature of 22.5°C.

Comparing the results with others from the sartorius muscle of the frog, the following differences have been noted: In the mammalian muscle the recovery heat starts early and is all over in about 5 minutes, thus contrasting with frog muscle in which the duration of the oxidative heat is about twice as great. The intensity of the process is thus much greater than occurs in frog muscle. In the case of a one second tetanus the recovery heat carries the galvanometer reading to a second maximum which is higher than that of the initial heat. The recovery heat for a series of twitches was about 1.5 times the initial heat and thus is of the same order as that reported by Hill and Hartree for frog muscle. On the other hand, for a one second tetanus the recovery heat varied between 2.5 and 3.5 times the initial heat.

These results indicate that at a given temperature the process of recovery takes place at an accelerated pace in mammalian muscle and suggest that the mechanism of recovery in the twitch may not be the same as it is in a tetanic contraction.

Some factors influencing glucose tolerance in the fasting dog. WILLIAM H. CHAMBERS and ELISABETH MARQUIS.

After performing between 100,000 and 200,000 kilogram-meters of work during a fast of 20 to 30 days the dogs temporarily lost the ability to oxidize ingested glucose but retained the power of glycogen formation. The administration of 50 grams of glucose caused a rise in blood sugar to over 300 mgm. per cent and an excretion of 5 to 10 grams in the urine. The condition was not corrected immediately by insulin, suggesting that the loss of carbohydrate stores by the tissues may decrease the oxidation of

¹ Richardson, H. B., E. Shorr, and R. O. Loebel, 1930. Journ. Biol. Chem., lxxxvi, 551.

² Shorr, E., R. O. Loebel and H. B. Richardson. 1931. This Journal, xcvii, 559.

sugar (Dann and Chambers). The following procedures were employed to reduce the carbohydrate content of the body with a shorter fasting period, using the glucose tolerance test and urinary excretion to indicate their value. A short fast of 4 or 5 days, both without exercise and when augmented by a transient diabetes from the injection of a gram of phlorhizin, gave no glycosuria with the tolerance test and a maximum blood sugar figure of about 185 mgm. per cent. Frequent intravenous injections of adrenalin aided rather than inhibited the glucose utilization. After a more rapid depletion of the glycogen stores by phlorhizin and strenuous exercise (200,000 to 500,000 kilogram-meters) during 8 days without food, the glucose tolerance curve was almost as high as that after the 3 to 4 weeks of fast. Heavy work for a shorter period (500,000 kilogram-meters in 3 days) was not so effective. These experiments indicate that the elimination of a large amount of sugar through a temporary glycosuria, combined with excessive muscular effort, is a factor of equal importance with the long fast in producing hunger diabetes.

The influence of ions on nerve respiration. T. H. CHANG, R. W. GERARD and MILDRED SHAFFER.

In previous work, we found that the respiration of frog nerves at 20° was alike in Ringer, pure NaCl, or 90 per cent NaCl containing also one or two of the salts KCl, CaCl₂, MgCl₂; and that this constant rate was maintained for twenty hours or more. These observations have been fully confirmed.

Phosphate buffer, however, was shown to cause a definitely increased respiration (15 per cent), and other calcium-ion removers—fluoride, oxalate, tartrate, citrate—have considerably greater effects, up to a doubling in sodium citrate. The effectiveness of these anions does roughly parallel valence; and this may play a minor rôle in the respiration increase, but it is surely not the main factor. Thus, for a series of sodium salts, respiration is changed from the value in the chloride as follows: Br⁻ = 0, I⁻ and CNS⁻ = +5 per cent, F⁻ = +25, NO₃⁻ = +30, SO₄⁻ = +10, HPO₄⁻ = +15, tartrate = +20, oxalate = +85, citrate = +105. Respiration increase does not follow the valence nearly so closely as the Ca⁺⁺ removing ability of the anion. Nitrate is probably the only aberrant ion in such a series. In the presence of extra calcium, citrate has no effect.

If Ca⁺⁺ removal, then, leads to respiratory increase, it was necessary to account for the equality of respiration in Ringer and saline. The clue came from studies on dog nerves at 38°C. Phrenic, sciatic, vagus and sympathetic chains were used, at first in phosphate-buffered Ringer. In all cases, after 3–4 hours during which oxygen consumption slowly diminished, there was a marked increase in respiration, sometimes to over doubling. This rise did not occur in serum, but in NaCl the respiration was high from the start and rose early to very large values. Adding calcium or magnesium ions to the saline largely or entirely prevented this, and in unbuffered Ringer the initial values were low and a subsequent rise very late and small, probably due largely, but not entirely, to loss of calcium by combination with nerve phosphates, etc. Addition of citrate to serum increased respiration. Frog nerves studied at 38°, and especially with the sheath split, gave similar results—a slow fall of respiration in Ringer or NaCl (with or without KCl) for 6–7 hours, followed by a marked rise in

NaCl, a slight rise or continued fall in Ringer. Typical results were: 2nd hour, Ringer = 135, NaCl = 165; 4th hour, 70, 95; 9th hour, 30, 310.

Average values for dog nerves in serum at 38°C., in oxygen for the early hours, in cmm. per gram and hour, were: phrenic 140, vagus 190, sympathetic 210. Br^{++} or Mg^{++} can replace Ca^{++} with nerve. Mg^{++} does not depress brain; Ca^{++} does.

Experiments with varying combinations and proportions of Na^+ , K^+ , Ca^{++} , Al^{+++} , and cane sugar cannot be detailed. Isotonic sugar give about a 30 per cent decrease of respiration, KCl 50 per cent, CaCl_2 50+ per cent. All but Ca^{++} were fairly easily reversible. Sugar with K^+ or Ca^{++} caused more decrease, sugar with Na^+ less decrease than sugar alone. Al^{+++} gave an immediate decrease of 65 per cent associated with coagulation.

These results are considered in relation to ion influences on other nerve attributes.

Comparative studies on the principles isolated from twelve species of toads.

K. K. CHEN, H. JENSEN and A. L. CHEN.

Some time ago we reported the results on an investigation of *Ch'an Su*. We have since been engaged in similar studies of the poisonous secretions obtained from twelve species of toads that were collected from different parts of the world. The venom was expressed from the "paratoid glands" and dried at room temperature. By the application of the usual methods of extraction, we have succeeded in isolating a large number of compounds that are physiologically active. The secretion of each species of the toads contains at least from three to five distinct principles that belong to the following five classes.

1. *Cholesterol* occurs in all of the secretions. Spectrographic examinations of several different samples showed the presence of ergosterol in various amounts.

2. *Epinephrine* is found to be present in five species.

3. *Bufagin* is a word used generically, since we have evidence that most of the bufagins, although similar in their action, are quantitatively different from each other in their potency. Besides, their physical constants and elementary composition are not the same throughout. The bufagins possess the essential features of the members of the digitalis group. The cat unit of each principle is less than 1 milligram.

4. *Bufotoxin* is a name used generically, for the same reason as given for bufagin. Chemically, the bufotoxins appear to result from the conjugation of one molecule of suberyl-arginine with one molecule of the specific bufagins found in the respective secretions. Their pharmacological action is similar to that of the bufagins, being different only in degree.

5. *Bufotenine* is also a word used generically. The bufotenines are organic bases containing an indole nucleus, and form organic salts, such as the flavianates which have been used in this investigation. Seven of the bufotenines have a marked pressor action in pithed cats.

The effect of acid feeding upon the composition of bone. A. L. CHUTE and LAURENCE IRVING.

The subject was investigated to see whether the bones participate in neutralizing ingested acid. Rats were fed on a diet acidified with HCl amounting to over 4 cc. of normal acid per 200 gram rat per day. The pH and titratable acidity of the urine increased and the blood alkaline reserve

was diminished. The muscles, as judged by CO_2 content, were unaffected. The femurs of the control series showed considerable variation in Ca, CO_2 and P. In the acid fed rats the bone minerals were also variable but in general indicated a depletion of these components as if they were being drawn upon for neutralization of the ingested acid.

The elasticity of the eyeball under different intraocular pressures. JANET H. CLARK.

In experiments reported in an article now in press (L. H. Weed, L. B. Flexner and J. H. Clark; This Journal, c, 246) pressures of cerebro-spinal fluid were measured by bubble manometer and by open-end manometers of various bores, in dogs of similar size. With larger open-end manometers the pressure alterations in the cerebrospinal fluid on tilting from horizontal to vertical positions, decreased in proportion to the amount of fluid dislocated into or from the manometer and there was found to be a constant relationship between the volume of fluid dislocated and the pressure change. This relationship, expressed in the fraction $\frac{dV}{dP}$, is a

function of the volume of the system and its elasticity. In animals of the same species the elasticity factor is constant enough to permit calculation of intradural volume changes from observed pressure changes.

Similar observations on the variation in volume with pressure have been made in the eyes of dogs and monkeys. By means of a bubble manometer the fraction $\frac{dV}{dP}$ has been measured and the results have been used to calculate the elasticity of the eyeball and to determine the pressure range over which the coats of the eye can function as elastic membranes.

The freezing-points of serum and corpuscles. DEAN A. COLLINS and F. H. SCOTT.

Previous investigators (Stewart, Moore and Roaf, Hamburger, Collip, Ege, Cherbuliez, and Koeppe) have found the freezing-point depression of the corpuscles to be less than that of their serum by about 0.040°C ., which corresponds to an osmotic pressure difference of about 400 mm. Hg. These differences are easily confirmed if certain precautions in the handling of the blood are not taken; while, if these precautions are observed, the freezing-points obtained for serum and corpuscles approach each other rather closely. A very large error results if the blood tubes are not kept stoppered during centrifugation. A surprising amount of evaporation takes place at the surface, which will affect the concentration of the serum more than that of the corpuscles. In one series of five experiments, in which the blood was centrifuged in open tubes, a difference of 0.051°C . was observed, the corpuscles having the smaller freezing-point depression. These results thus confirm those of the previous workers. However, when the same bloods were centrifuged in closed tubes, the average difference from the five experiments was only 0.001°C . Another possible source of error is the unequal escape of carbon dioxide from cells and serum. We give the following average differences compiled from more than fifty experiments. In some of these experiments an attempt was made to limit the carbon dioxide loss, and in all of them the blood was centrifuged in stoppered tubes.

KIND OF BLOOD	DIFFERENCES	METHOD OF CO ₂ CONTROL
	°C.	
Beef (very venous)	0.014	None
Dog (arterial)	0.004	None
Beef	0.006	The blood was equilibrated with room air before centrifugation
Dog	0.002	
Beef	0.012	The blood was in equilibrium with and all manipulations were made under alveolar air
Dog	0.002	
Beef	0.008	The procedures were carried out under paraffin oil
Dog	0.008	

While the unequal escape of gas may have some effect, it is not nearly as important as improper centrifugation.

It should be noted that undercooling is especially important in a freezing-point determination when the material contains large amounts of solids. A special formula taking this fact into account has been worked out by one of us.¹ Correction for undercooling in our experiments has been made by the use of this formula.

When the blood is properly handled and the proper correction for undercooling made, the differences between the freezing-points of serum and corpuscles become so small that we hesitate to regard them as significant.

The production of experimental convulsions in the white rat. HELEN C. COOMBS.

In a comparative study of the effects of convulsant drugs, e.g., absinth and camphor monobromide, a series of experiments was done on adult albino rats. After tying the animals out and injecting Scarpa's triangle with novocain, carefully calibrated doses of a standard solution of camphor monobromide were injected into the femoral vein, and the effects studied.

In control animals the results were as follows:

1. Sub-minimal convulsive doses of camphor monobromide produce restlessness, straggles, clonic twitches and rapid respiration.

2. The minimal convulsive dose of the drug produced a clonic convulsion comparable to that shown by the cat, and of somewhat longer duration—about three to five minutes. The average minimal convulsive dose was about 1 mgm. of camphor monobromide per 100 grams body weight, or 4.5 mgm. per pound, whereas in cats the average minimal convulsive dose is about 2.2 mgm. per pound.²

3. The recovery period between injections of the convulsant drug is about twenty minutes. If one injection follows another too rapidly, there may be no convulsive response to the second dose, or the animal may succumb without another clonic convulsion, but with movements of tonic extension only.

¹ Collins, D. A., Proc. Soc. Exp. Biol. Med., 1931, xxviii, 896.

² Wortis, S. B., H. C. Coombs and F. H. Pike, Arch. Neurol. and Psychiat., 1931, xxvi, 131.

Pike, F. H., C. A. Elsberg, W. S. McCulloch, and A. Rizzolo. Amer. Journ. Psychol., 1929, ix, 259.

Coombs, H. C., This Journal, 1932, c, 65.

4. No sweating of the feet was observed at any time. Owing to the albino eyes, it was not possible to get an idea of the relative dilatation of the pupil. On post mortem, the stomach and large intestine were ballooned up, as in the cat.

5. Electrical stimulation of the cortex produced clonic movements during the period of stimulation, but there was little or no persistence of the clonic convulsion after the cessation of stimulation.

The effect of cortico-adrenal extract on the blood-cellular elements. E. L. COREY and S. W. BRITTON.

Observations have been made on the blood changes produced in adrenalectomized and extract-treated rats and cats. Controls were injected with saline and also with adrenalin in concentration similar to that found in the extract. The neutrophilic and lymphocytic counts were found to be chiefly affected. Following extract injection, the neutrophils rose between 100 and 200 per cent, while pronounced decreases in lymphocytic counts were concurrently observed. Somewhat similar changes were found to take place, however, on the administration of adrenalin. Other cellular elements of the blood did not fluctuate beyond the normal variations.

Maternal leucocyte changes following fetal death. E. L. COREY.

In a series of rats fetal death was brought about by mechanical separation of the placenta from the endometrium. Total and differential leucocyte counts were made before and for 10 days subsequent to operation. In two control animals, dummy laparotomy operations were performed. Two of the experimental animals died after 1 and 3 days respectively. In these animals the fetuses measured approximately 30 mm. in crown-rump length, in contrast to those in the remaining animals which measured about 20 mm. The early death of these animals may be attributed to the considerable toxicity of the large fetuses in utero. The neutrophile count showed an abnormally high level in these cases. In the remaining animals the blood elements exhibited no changes which differed in any marked degree from the controls. Curves representing the cellular constituents of both experimental and control animals paralleled each other throughout the period of experimentation. The total leucocyte counts for both groups were practically identical. In a second series of pregnant animals laparotomy was performed, the uterus incised and the contained fetuses removed and immediately placed free within the peritoneal cavity. Total and differential counts were then made daily for 10 days. In these animals the neutrophile count rose rapidly and maintained its high level throughout the experimental period. A similar rise was noted in the total white cell count. It is suggested that the uterus may therefore act in a protective capacity against the toxic substances liberated by the necrosing (resorbing?) fetuses, while the dead fetal tissue within the peritoneal cavity reacts in an irritating and toxic manner, probably more particularly as a foreign body. The process of maceration or resorption of dead fetuses in the white rat does not appear, therefore, to involve leucocytic intervention.

A possible correlation between early ossification and the blood elements of the albino rat. E. L. COREY.

In former studies Kindred and Corey have reported the normal total

red and white cell content together with the hemoglobin values of the blood of albino rat fetuses during the latter third of the gestation period. In all of these elements marked changes were observed to take place at approximately the 23 mm. stage. Thus, the white cell count reached its highest pre-natal value (with the exception of counts made shortly before birth) at this period, and the red blood cells showed a high peak at this stage. A series of albino rat fetuses was fixed at different age periods and the bony elements stained after the alizarin method of Dawson (1927). This method allows a thorough study of the early ossification centers, necessitating very little dissection. On studying the series it was noted that the earliest centers of ossification observable appeared at a crown-rump length of 22.7 mm. The centers observed were mainly in agreement with those described by Strong (1925). Hence a possible correlation between the beginning of ossification and changes in the blood elements is suggested.

Observations on circulation in the fetal albino rat. E. L. COREY.

Observations were made on the heart rate of 138 fetuses from 8 mm. crown-rump length until term. Twenty-one litters were used. Considerable variation in cardiac activity in members of the same litter was noted. Averages of the heart rate of male fetuses were slightly in excess of those for females. The lowest average heart rate occurred in fetuses of 8 mm. crown-rump length (117 beats per minute). From this point onward the average rate rose to 197 beats per minute at the 16.4 mm. stage. Following considerable fluctuation, the heart rate rose to 223 beats per minute at 23.3 mm. Decreasing at this point, a third sharp rise to 243 beats occurred at 34.2 mm. (the highest average rate recorded). At term the heart rate averaged 185 beats per minute. Curves representing the fetal and maternal heart rates presented a definite correlation, paralleling each other closely without exception. The maternal heart rate was higher, in all cases, than that of the fetuses in utero. Vagal stimulation in the mother failed to affect the fetal heart. By means of timing the rate of flow of India ink placed with the ventricle of the fetal heart the approximate blood velocity in 62 fetuses, comprising 13 litters, was determined. It averaged 15 mm. per second. The resulting curve was similar to that representing the heart rate of fetuses of the same crown-rump length. As a further check on the above observations, the blood pressures of 16 fetuses, from 4 litters, were determined. The pressures recorded were in keeping with the previous results. When all data obtained were compared, three distinct rhythms in circulatory functions appeared to be present. The periods of greatest activity occurred at approximately the 17, 23 and 35 mm. stages.

Blood volume after intravenous fluid in normal and Eck fistula dogs. LATHAN

A. CRANDALL, JR. and GEORGE M. ROBERTS.

Poindexter and Miller¹ have shown that 0.9 per cent sodium chloride solution amounting to as much as 30 per cent of the body weight may be given to normal dogs intravenously at the rate of 100 cc. per minute without increasing the blood volume measured by the dye method. Hematocrit, erythrocyte count, and plasma proteins show the expected change but the blood volume remains constant even though the determination is made within 1 to 2 minutes after completing the injection of saline. We have

¹ Poindexter, C. A. and J. R. Miller. Journ. Lab. and Clin. Med., in press.

repeated and confirmed these observations, and further, we have found that the same phenomenon follows the injection of 5 per cent dextrose. It occurred to us that these findings might be explained by a storage of blood in the vessels of the splanchnic area; therefore we have repeated the procedure on Eck fistula dogs.

A series of 34 blood volume determinations, using brilliant vital red, has been made on five Eck fistula dogs before and after the intravenous injection of 50 to 100 cc. of isotonic saline or glucose per kilo, the rate of injection being approximately 100 cc. per minute. The interval between completion of the injection and injection of dye has varied from 1 to 15 minutes. In four of these animals the intravenous injection of fluid invariably increased the blood volume as measured by the dye method, the increase usually accounting for 40 to 50 per cent of the injected fluid. The cell volume usually somewhat decreased, occasionally slightly increased or not changed; the increase takes place entirely in the plasma volume. In normal dogs in which the total blood volume is not changed there is a definite increase in plasma volume and equal decrease in cell volume, indicating either that both cells and plasma are leaving the circulation, or that diluted blood is being held in the vessels but not actively circulating. The fact that it is possible to increase circulating blood volume in Eck fistula animals by intravenous fluid supports the latter hypothesis.

The fifth Eck fistula dog never showed an increase in blood volume, nor did this animal exhibit the increased plasma volume which is found in the majority of normal animals; the passage of fluid out of the vessels seemed to be more rapid in this animal than in most normal dogs. Two splenectomized dogs gave results entirely comparable to those of normal animals.

The influence of certain conditions in the duodenum on the rate of secretion and acidity of the gastric juice. J. O. CRIDER and J. EARL THOMAS.

This study was made on three dogs, each with a Pavlov pouch and a permanent duodenal fistula. The pouch secretion due to a standard meal of raw lean beef was collected directly into 10 ml graduated cylinders in amounts of 1 to 4 ml each. The time required for the collection of each successive ml was noted and the acidity of each sample determined by titration. Besides normal controls, experiments were conducted during which tenth normal HCl was injected into the duodenum at the rate of approximately 4 ml per minute for two hours, beginning between thirty and forty-five minutes after feeding, and experiments during which the duodenum was drained to the outside.

During the first hour of the injection of HCl the rate of secretion, and generally the acidity, were less than during the corresponding phase of the control experiments. During the latter half of and following the injection period the rate of secretion averaged higher than normal.

Continuous drainage of the duodenum, beginning at the time of feeding, caused a sharp and permanent decline in the rate of secretion after the first half hour, as compared to the normal, and reduced the volume secreted in a four hour period to about one-third of the normal. Replacing the lost fluid by intravenous injection of Locke's solution (without dextrose) failed to alter materially this result.

Assuming that drainage of the duodenum merely abolishes the "intestinal phase," the results indicate that this phase is responsible for a far larger proportion of the total gastric secretion than is manifest from previous work. The investigation is being continued.

Further studies of autotrophic cells. GEORGE CRILE, OTTO GLASSER, MARIA TELKES and AMY F. ROWLAND.

Cultures continued for ten months showed no variations in physical characteristics of successive generations of cells. Physical and chemical phenomena incident to activities of cells will be described and also the effect of anesthetics, narcotics, etc.

The effect of anoxemia on the emptying time of the stomach. GEORGE CRISLER and EDWARD J. VAN LIERE.

Dogs were fed a meal consisting of 40 grams of hamburg, 10 grams of dry bread crumbs and 15 grams of barium sulphate mixed with 50 cc. of milk. The normal emptying time of the stomach was established fluoroscopically previous to the experimental period on several different days. During the experimental period the emptying time was determined under anoxic and under normal conditions on alternate days. Anoxemia was produced by placing the animals in a steel tank and exhausting enough air to subject them to varying pressures (constant in any given experiment) down to $\frac{1}{2}$ atmosphere. Ventilation was controlled by an intake valve which allowed air to enter the tank in a constant stream but only fast enough so that the pressure could be maintained with the pump. This stream of air was sufficient in volume to prevent accumulation of carbon dioxide. The emptying time was prolonged, in many cases being doubled.

An electrocardiographic study of viscerocardiac reflexes. P. J. CRITTENDEN.

This work is a part of the study of viscerocardiac reflexes, which has been under investigation in our laboratory (Ivy). In this work the question of the rôle of nausea, vomiting and jaundice was undertaken. Of thirty unanesthetized animals receiving apomorphine subcutaneously, eight showed heart block, six, cardiac arrest. Various changes occurred in the form of the various electrocardiographic waves. Prior to the onset of vomiting, the heart rate was increased. Just before and during the vomiting act, the heart rate was markedly decreased.

Atropine *per se* intravenously markedly increased the rate in fourteen animals. When apomorphine was given to eleven of the above animals, heart block and cardiac arrest noted above did not occur, and nausea, retching and vomiting caused much less variation in the rate. Hence the nervous influences which produce cardiac irregularities after apomorphine pass to the heart through the vagi.

In fourteen animals jaundice seemed to increase the susceptibility of the heart to cardiac arrests, heart blocks and similar phenomena induced by the action of apomorphine.

In sixteen animals, jaundice *per se* caused the appearance of interpolated beats in two, "f" waves in one, and also the disappearance of "spontaneous" heart block in two. The rate was increased in eleven, decreased in four and in one there was no change.

In five jaundiced dogs, distention of the biliary system causing nausea, vomiting, retching and pain, affected the animals differently. Abnormal beats, changes in R voltage and heart rate occurred. The changes seemed to parallel the degree of jaundice.

In 92 students, swallowing of the stomach tube caused primarily an increase in rate, and in eight of these abnormal beats occurred.

On the existence of afferent respiratory impulses mediated by the stellate ganglia. S. P. CROMER and R. H. YOUNG.

After observing respiratory death in twenty dogs on central stimulation of vagi after removal of the stellates (A. C. Ivy and S. P. Cromer), we became convinced that the stellates mediate impulses that affect the respiratory center. In this connection the work of D. T. Barry, E. H. Craigie, O. Larsell, and G. E. Burget on afferent pulmonary impulses seemed to be worth repeating with our technique.

Barry clamped the trachea of rabbits at the end of inspiration as well as at the end of expiration and stated that there were probably inspiratory impulses passing via the stellates to the medulla, while inhibitory impulses passed over the vagi. On repetition of Barry's work, using eight dogs, practically uniform results were obtained, showing that the pattern of response to the clamping stimulus was different after stellectomy. It is doubtful that the changes seen were due to the removal of inspiratory impulses.

Craigie introduced irritating vapors (ammonia and ether), into the lungs of dogs, and concluded that afferent impulses traveled over the sympathetics via the cord to the medulla, since sectioning of the vagi had no effect. Larsell and Burget, with similar technique on rabbits, and a few dogs, concluded that the afferent impulses were mediated by the vagi alone.

Using a similar method on five dogs we find that impulses incited by ammonia vapors travel equally over the vagi and the sympathetics.

A study of the conversion of fat to carbohydrate in the germinated castor bean by means of a modified oxycalorimeter. R. G. DAGGS and H. C. H. WARDLAW.

By combining the Benedict and Fox¹ oxycalorimeter and the Benedict Universal metabolism apparatus, and introducing a more effective cooler, one is enabled to obtain accurate respiratory quotients upon the combustion of small amounts of dried plant or animal material. By burning castor beans at different stages of germination, one has indication of the type of material present. This in conjunction with the chemical analyses of the beans and the respiratory metabolism of the living beans, which has been done in this laboratory, will prove whether or not the fat of the castor bean is converted to sugar in the formation of new plant cells.

By burning a pure substance such as sucrose, one can obtain an actual R. Q. that checks very well with the theoretical, thus demonstrating the efficiency of the set up. The average R. Q. for the ungerminated bean is 0.75, which agrees with the average chemical composition. Upon germination the R.Q. rises indicating the decreasing percentage of fat and the increasing percentage of carbohydrate. When the radicle has attained a length of 30 mm. the R.Q. has changed from the 0.75 of the ungerminated condition to 0.81. The R.Q.s of all stages of germination up to a radicle length of 10 cm. are being determined.

Respiratory metabolism during premortal rise in nitrogen excretion. MARGARET DANN.

A mongrel female dog weighing 16 kilos was fasted until death on the 36th day. Its fat depositories were obviously depleted. During this

¹ Journ. Biol. Chem., 1925, lxvi, 783.

period it performed about 360,000 kilogram-meters of work on the treadmill and lost about 50 per cent of its weight. On the 32nd, 33rd, and 35th days of fast the average respiratory quotients were 0.79, 0.84, and 0.79, respectively, in marked contrast to R.Q.s of 0.71 or 0.72 repeatedly found in dogs fasting 20 to 30 days. The explanation of the high R.Q.s appeared in the fact that the dog excreted 0.209, 0.527, and 0.378 gram of nitrogen per hour on these 3 days, which accounted for 37, 100, and 80 per cent, respectively, of the total calories. The close agreement between the indirect heat (15.12, 13.97, and 12.52 calories per hour for the 32nd, 33rd, and 35th days) and the heat directly measured (16.08, 14.19, and 12.91 calories, respectively) showed that the dog was apparently able to oxidize completely the products of the breakdown of body protein. In contrast to this, previously reported experiments showed that neither ingested sugar nor the sugar fraction derived from ingested protein is oxidized by dogs fasted 20 to 30 days. In a series of 21 experiments on 12 dogs following prolonged fasts, only one showed any indication of "premortal rise" in nitrogen, and in it protein accounted for 36 per cent of the calories of metabolism. In the other 20 the variation was from 7.4 to 22.0 per cent and averaged 13.8 per cent of the total calories produced.

The effect of alcohol on the electric mobility of gelatin. JANET DANIEL and H. A. ABRAMSON.

By studying the electric mobility of quartz particles covered with a gelatin film, it was possible to study the effect of alcohol on the electric mobility of the protein up to 60 per cent ethyl alcohol in conjunction with the titration curves of gelatin in the same media. It was found that the isoelectric point of the protein-covered particles was pH 5.3 in 35 per cent alcohol (vol. per cent) and pH 5.8 in 60 per cent alcohol. By interpolation experiments over this range the change in pH of the isoelectric point was found proportional to the change in the dielectric constant of the medium, neglecting the effect of salts. In harmony with our previous rule, it was found that in any given concentration of alcohol, the mobility was roughly proportional to the acid bound by the protein. In different concentrations of alcohol no simple relationship has as yet been found.

As previously found for aqueous systems the factor of proportionality for electrophoretic and electro-osmotic mobilities in these alcoholic media was 1.00, very nearly in accord with the theory of von Smoluchowski and Henry, and contrary to the point of view of Debye and Hückel.

The frequency of impulses in the auditory pathways. H. DAVIS and L. J. SAUL.

Electrical auditory responses have been traced from the VIII nerve to the auditory radiations. These responses, when amplified, reproduce in our telephone receivers, with fair approximation, sounds applied to the cat's ear. At least two effects must be distinguished: true nerve action currents, and an electrical "spread" from the region of the cochlea.

The "spread" is diffuse. It can be picked up from any tissue of the head. It reproduces with great accuracy the sound patterns applied to the ear. With our present apparatus we hear it best at frequencies between 1000 and 3500 vibrations per second. It may persist for many minutes after heart and respirations have ceased.

Action currents, on the contrary, have the following characteristics.

They are very sharply localized to the auditory tracts. They follow the known contralateral relationships of these pathways. They are reversibly deleted by narcotics, and disappear immediately at death. Larger excursions may be obtained on a string galvanometer from the central tracts than from the VIII nerve. Musical notes of moderate intensity are most clearly recovered from the central tracts at frequencies between 200 and 1000 per second. Words spoken to the cat's ear are unintelligible unless the action currents are reinforced by "spread." The details of speech, particularly consonants, are lost, probably because frequencies above 1000 per second are not reproduced. When, as is most marked in leading off from the VIII nerve, action currents and "spread" are simultaneously present, they may be distinguished by the administration of ether or chloroform. This gives a differential and reversible suppression of the frequencies below about 1000 per second.

We conclude tentatively that the highest frequency of nerve impulses in the fibers of the auditory pathways does not exceed 1000 per second.

Observations on an anaphylatoxin in anaphylactic shock. CARL A. DRAGSTEDT and ERICH GEBAUER-FUELNEGG.

Evidence is presented that in anaphylactic shock in the dog there may appear a principle or substance in the inferior vena cava blood or the thoracic duct lymph capable of producing smooth muscle contraction in the surviving guinea-pig intestine. Experience to date indicates that the occurrence of this substance is not invariable, occurring in approximately 40 per cent of our experiments. The evidence so far obtained indicates that the substance occurring in the inferior vena cava blood is identical with that appearing in the thoracic duct lymph. The substance appearing during shock has the same properties irrespective of the antigen used. Observations on the pharmacological and chemical properties of the active substance are reported. There are indications that it is a simple chemical entity.

The cause of death in experimental acute pancreatitis. LESTER R. DRAGSTEDT and H. E. HAYMOND.

Experimental evidence is presented which indicates that the simple activation of pancreatic enzymes within the pancreatic ducts is not sufficient to cause digestion of the pancreas.

Pancreas implanted into defects in the duodenum is not digested by the active trypsin and erepsin of the duodenal content. Isolated areas of the pancreas of the dog are not digested when immersed in activated dog's pancreatic juice for six hours. The legs of living frogs are not digested if immersed in active pancreatic juice of the dog. It is probable that the local toxic action of bile salts produces necrosis of the pancreas and that the activation of the trypsin in the gland is of secondary importance. The necrotic pancreas is broken down more rapidly than the other tissues because of the activated trypsin. The uncontaminated pancreas of normal dogs regularly contains both aerobic and anaerobic bacteria. These bacteria are of predominant importance in the genesis of the toxemia of acute pancreatitis, either through their manufacture of a specific toxin or more probably through the production of toxic protein derivatives, as a result of their metabolism or action on dead tissue.

The in-vivo autolysis of uncontaminated pancreas in the presence of

the bacteria normally present produces a severe toxemia and death. The in-vivo autolysis of pancreas made sterile by autoclaving at 15 pounds' pressure for fifteen minutes does not produce a toxemia. The in-vivo autolysis of pancreas, made sterile by autoclaving as before but mixed with active pancreatic juice previously sterilized by Berkefeld filtration, does not produce a toxemia if placed in the free peritoneal cavity. The in-vivo autolysis of fetal pancreas, proved sterile by culture, does not produce a toxemia. The in-vitro digestion of sterilized pancreas, liver, or muscle by pancreatic or gastric juice, in turn sterilized by Berkefeld filtration, does not produce a toxic product if bacterial contamination be avoided.

Experimental pancreatic necrosis in the dog is accompanied by changes in the blood chemistry similar to those occurring in high intestinal obstruction.

Observations on the lymphatics in the web of the frog. CECIL K. DRINKER and MADELEINE E. FIELD.

1. A description of new methods for observing the lymphatic capillaries.
2. The nature of the lymphatic endothelium.
3. The comparative extent of the absorbing surface offered by blood and by lymph capillaries.
4. The absence of contractile power in the web lymphatics.

Significance of variation in basal metabolic rate determinations. HALBERT L. DUNN and WALTER M. BOOTHBY.

A statistical study will be presented to show the effect of the following elements upon the basal metabolic rate: *a.* The variation of the rate of heat production in an individual as affected by menstrual cycle, diurnal trend, seasonal trend and dietary shifts. *b.* The type and magnitude of the variations found within the day as compared with the variations over long periods, such as a month or year. *c.* The type and magnitude of the variations between individuals.

The release of a copulation reflex pattern in the "secondary" spinal cat. J. G. DUSSER DE BARENNE and Y. D. KOSKOFF.

The pattern of the "decapitate" cat is well known. The muscles of the limbs do not show marked tension or "tone," and the limbs remain in any position which is given to them.

The picture after "secondary" decapitation, that is, when the decapitation is done after primary decerebration, is entirely different.

In such a preparation a strong, springlike flexion of both hindlegs with strong priapism develops when the animal is in prone position. Dorsiflexion of the tail nearly always augments the flexion of the hindlegs and the priapism and often results in ejaculation and emission of small jets of urine. This syndrome has been observed to persist as long as 24 hours.

The frontlegs are usually flaccid. Sometimes a definite resistance against extension and abduction can be observed in these limbs.

Subsequent "tertiary" transection of the spinal cord at the level of the first lumbar segment is often followed by a marked augmentation of this flexion posture and priapism. The tension to overcome this flexion is often very large, 1100 grams and more. Also in the spinal preparation, with secondary transection of the cord at L₁, this flexor rigidity with priapism appears.

Section of the lumbosacral posterior roots of one side abolishes the flexion rigidity in the homolateral hindleg; it is, therefore, a reflex phenom-

enon. If the flexion rigidity is overcome by forceful extension of the leg, lasting a few seconds, the hindleg returns with a quick springlike movement into the flexed position. If the extension is maintained for several seconds, 8 to 12, the leg remains extended. On stimulation of the skin of the hindleg or of the ano-genital region it immediately reassumes the flexed position.

This syndrome is interpreted as a copulation reflex pattern released after "secondary" decapitation.

The absorption of insulin from the gastro-intestinal tract. A. G. EATON and JOHN R. MURLIN.

Totally depancreatized dogs are able to absorb considerable insulin when given with calcium lactate and sodium bicarbonate. The blood sugar falls 40 to 95 mgm. per cent, the respiratory quotient rises and the urine usually becomes sugar-free for 4 to 8 hours after administration. The calcium lactate and sodium bicarbonate are believed to act as buffers, removing the free hydrochloric acid from the stomach and protecting the insulin from destruction by pepsin. When pancreatin is given with the food, the absorption is much less. A preliminary trial on three human diabetics showed a fall in blood sugar of about 60 mgm. per cent in one case, and none in the others.

Measurements on the visco-elastic changes in muscle under pressure. DAYTON J. EDWARDS and MCKEEN CATTELL.

A strip of ventricle from the heart of the terrapin was used in some instances and a pair of sartorius muscles in others. The muscle was attached by one end to an optically recording torsion wire lever and by the other to a small rod. A release device permitted a quick shift of the rod either up or down and this in turn caused a greater or a less stretch upon

TABLE 1

PRESSURE	TIME						
	Beginning	0.04 second	0.08 second	12 seconds	16 seconds	20 seconds	24 seconds

A. Quick stretch of sartorius

pounds	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2,000	5.6	3.6	3.8	3.3	3.0	2.8	2.6
4,000	9.8	7.3	6.2	6.8	6.5	6.6	7.0
6,000	17.6	14.7	16.2	15.2	15.6	14.4	13.3

B. Quick release of sartorius

2,000	13.2	12.9	11.3	10.2	9.9	9.9	9.9
4,000	29.6	24.2	22.1	21.5	21.5	21.5	21.5
6,000	67.2	48.4	40.0	37.1	35.0	34.6	34.2

the muscle. By taking a record of the movement of the lever while a quick stretch or release was given to the muscle, curves were obtained which show typically a sudden shift of the position of the lever followed by some highly damped vibrations and then a period of "sink" to finally reach a new equilibrium. The contour of the curves from muscle preparations, when free from pressure and when subjected to pressure, give the data for this study.

The results given in table 1 are average values computed from 8 experiments. Measurements were made of the height to which the lever was carried immediately when stretch or release was given and at subsequent intervals of 0.04 second. The curves obtained from muscles subjected to pressure have been related to control curves and the results expressed on a percentage basis. The first column of part A of the table shows that pressures from 2000 to 6000 pounds produce increases of from 5.6 to 17.6 per cent in the property of the muscle concerned with the immediate transmission of a strain. In a similar way the data in the first column of part B show that a muscle under a slight initial tension transmits the change of release more completely when under pressure. The increase in the immediate transmission of a change in tension by a compressed muscle denotes an increase in the rigidity of the muscle constituents. In tests with a rubber elastic system we have obtained a similar type of effect of pressure. The data in the last column of the table give the approximate new level of equilibrium and they show that the higher the pressure used the greater this level becomes raised over the control curve. This change gives additional proof of an alteration in the elastic property of the muscle.

A second change in muscle as a result of pressure appears as a retardation in the rate of decline to a new equilibrium following a quick stretch or release. The values in column 2, and to a less extent in column 3, bring out the fact that in both the release and the stretch experiments the muscle exhibits a brief period of recoil after a sudden change in its state of strain and that this process of recoil becomes slowed up by pressure with the greatest effect shown in the 6000 pounds' range. These results point clearly to an increase in the viscous property of the muscle under pressure.

Glycogen storage by fresh-water mussels. M. M. ELLIS and D. B. CALVIN.

Quantitative studies of glycogen storage in 90 animals representing six species of fresh-water mussels, following various control feedings have been made. All mussels were reduced to a fasting metabolic level before feedings were given. During the two-hour feeding periods the individual animals were placed in five liters of tap water containing the test substances. Throughout the experiments temperature, pH, dissolved oxygen and carbon dioxide were properly controlled. The condition of each animal was checked by continuous activity records and by studies on the cardiac efficiency at the end of the feeding period. As there is evidence of direct absorption of food material in solution by mollusks both suspended and dissolved substances were tested.

The results for the species studied may be summarized as follows:

1. Pronounced glycogen storage in both the hepato-pancreas and the pedal musculature of fresh-water mussels was found following feedings with either starch suspensions or dextrose solutions.
2. Small glycogen storage followed feeding with a suspension of cooked hen's egg yolk.
3. Little or no glycogen storage followed feeding with suspension of cooked egg white.
4. Feedings of glycerine seemed detrimental as there were actual glycogen losses in the animals exposed to this compound.
5. Feedings with solutions of ammonium tartrate produced glycogen storage. This was of particular interest in view of the use of this compound as a basic food.

All glycogen determinations were made on animals previously frozen with carbon dioxide snow. Modification of the Sahyun and Alsberg technique was used.

The effect of respiration on cardiac output. J. A. E. EYSTER and EARL V. HICKS.

In dogs under anesthesia in which stroke volume and minute cardiac output from the two ventricles is measured by a cardiometer during normal breathing, there occurs during inspiration a diminished stroke volume and a slight fall of right auricular pressure. The effective venous pressure is increased, since the fall in auricular pressure is not as great as the fall in intrathoracic pressure. The diminished total stroke volume may perhaps be explained on diminished left ventricular output due to retention of blood in the increased pulmonary vascular bed.

In the greater reduction of intrathoracic pressure associated with the deep inspirations following vagus section, these effects are exaggerated and in addition the diastolic volume is increased.

Under the conditions of these experiments, marked alterations of breathing have little effect on the average stroke volume or on minute volume when considered over a period of time. It is believed that the influence of the extent of breathing on venous return is not as great as is ordinarily stated.

Hemodynamics of arteriosclerosis: effects of increase in coefficient of volume elasticity of large arteries on circulation. GEORGE FAHR, JAY DAVIS, ARTHUR KERKHOF, PHILLIP HALLOCK and ELLIS GIERE.

In order to study the effect of arteriosclerosis of the large arteries on the work of the heart, the minute volume and the blood pressure, the following artificial vascular system was connected to the descending aorta and the superior vena cava of the heart-lung preparation of Starling.

A thin walled rubber tube 100 cm. in length and 12 mm. in diameter kindly constructed by the Research Department of the Goodrich Rubber Company according to our specifications is enclosed within a lead pipe 35 mm. in internal diameter. The rubber tubing has a coefficient of volume elasticity about two-thirds that of the normal human artery as determined by A. V. Hill. The lead pipe has six stopcocks fitted into it. When the space surrounding the rubber tube within the lead pipe is filled with water and the stopcocks closed, the coefficient of volume elasticity of the tube is approximately that of water, i.e., very rigid. When the stopcocks are opened the coefficient of volume elasticity of this large artery system is approximately that of the rubber tubing. In other words, by merely closing the stopcocks we can at will produce a very rigid large artery system out of a large artery system of slight rigidity.

The large artery system is connected to the heart lung preparation at the aortic end by a glass cannula of 10 mm. bore. The other end is connected to the artificial resistance of the heart lung preparation. This latter represents the small arteries and capillaries of the dog's systemic circulatory system. From the artificial resistance blood flows into the venous reservoir and the superior vena cava. All the blood leaving the left ventricle excepting that flowing through the coronary arteries flows through the above artificial circulatory system into the right auricle.

The heart was enclosed in a Henderson type cardiometer to record volume

changes of the ventricles. Stroke and minute volumes could be determined from this volume change. The minute volume of the systemic circulation minus the coronary flow was measured by means of a stop watch and measuring cylinder. Blood leaving the end of the rubber tubing and entering the venous reservoir could be diverted into the cylinder at will and measured. Blood pressure was measured with a mercury manometer in all experiments excepting a few where an optical manometer of very high frequency (Wiggers' construction) was used in order to obtain accurate pressure records. The coefficient of volume elasticity of the large artery system plus the small artery and capillary system varied from 70 per cent to 700 per cent of coefficient of normal human arterial system as determined by A. V. Hill. Working with low, moderate and high blood pressure and with small, medium and large minute volumes the results were always the same. The pulse pressure increased 100 per cent in arteriosclerosis, the arithmetical mean pressure dropped because the diastolic pressure dropped more than the systolic rose. The diastolic volume of the heart usually decreased slightly showing that the total energy consumption of the heart was less in arteriosclerosis of the large artery system.

The volume velocity of flow in the systemic system remained about the same in arteriosclerosis as in the normal. The minute volume dropped a little, showing a decreased coronary output due to decreased diastolic pressure.

The stimulating effect of carbon monoxide on muscle metabolism. W. O. FENN.

The inhibiting effect of CO upon the metabolism of yeast has been cited by Warburg as evidence in favor of the theory that some iron compound serves as the catalyst for oxidations in tissues. No such inhibition occurs however in frog sartorius muscles where, for example, a mixture of 79 per cent CO + 21 per cent O₂ gives a rate of oxygen consumption of about 1.4 cu. mm. per gram per minute while the matched muscle in air consumes at a rate of 0.6 cu. mm. per gram per minute. This effect is reversible and is independent of light. It is observed also in muscles poisoned with bromacetic acid, isotonic, KCl and isotonic glucose, in muscle mash, in frog heart and liver, in rat liver, muscle and heart, but not in frog kidney, skin or nerve. No effect upon the fatiguability, or the length of the muscle could be established in gastrocnemius muscles. CO had no effect upon the pH as judged by the CO₂ content at a given CO₂ tension. The rate of anaerobic lactic acid formation was the same in CO as in N₂ as judged by the CO₂ liberated. The phosphocreatin breakdown was the same in 10 per cent CO₂ + 90 per cent CO as in 10 per cent CO₂ + 90 per cent N₂ as judged by the rate of CO₂ absorption. The ratio of initial heat to tension time for a short tetanus was not significantly influenced by CO. The ratio of total heat to initial heat for a short tetanus was decreased by CO from 2.76 to 1.77 as judged by measurements of heat directly and from 2.59 to 1.89 as judged by measurements of excess oxygen consumption. Irritability is unaffected by CO.

The production of functional corpora lutea unilaterally by the direct intra-follicular injection of extracts of urine of pregnancy. MAURICE H. FRIEDMAN.

In earlier experiments it was found that follicles injected with small

amounts of extracts of urine of pregnancy developed into corpora lutea in the absence of any general humoral change of sufficient magnitude to produce any discernible change in the ripe follicles in the contralateral, untreated ovary. All attempts to demonstrate some functional activity on the part of these unilateral corpora lutea, however, proved futile. The present experiments were designed to discover the reasons for the lack of functional manifestation on the part of these unilateral lutein bodies.

Briefly, the results of the present experiments may be summarised as follows:

1. In about one-third of the animals in which the contralateral (untreated) ovary was removed 24 hours after the intrafollicular injection, the unilateral corpora lutea so produced proved to be functional (decidual reaction).

2. The incidence of functional corpora lutea was equally as great, however, in a series of animals in which the untreated ovary was left in situ.

3. It is evident, therefore, that the presence or absence of the untreated ovary is not of great significance in this regard. Moreover, if there exists a physiological antagonism between the secretion of ripe follicles and the secretions of corpora lutea, such antagonism was not evident under the conditions of these experiments.

4. From a series of control experiments it is apparent that the failure to secure a greater percentage of functional corpora lutea is to be attributed to the operative procedures. Hence, it is probable that the better results obtained in these experiments as compared with those of last year are to be credited to an improvement in technique which the operator himself did not appreciate.

An examination of cerebrospinal fluid, by means of the uterine fistula, for the presence of the oxytocic factor of the posterior pituitary. GERTRUDE SANDERS FRIEDMAN and MAURICE H. FRIEDMAN.

The use of the uterine fistula technique for the detection of the oxytocic factor of the posterior pituitary removes the difficulties inherent in the isolated strip method. The fistula preparation will not respond to the intravenous injection of non-specific fluids or proteins, nor to the injection of calcium salts histamine (except possibly, in large amounts—40 mgm. and 0.5 mgm. respectively). The isolated strip, on the other hand, responds to minute quantities of any of these in a way indistinguishable from its response to pituitrin. The intravenous injection of cerebrospinal fluid obtained by cisternal puncture from normal dogs has never given the slightest response in the fistula. Yet, the sensitivity in each case was such that a definite response should have been obtained even if the C. S. F. contained only the lowest concentration given by Dixon for the oxytocic factor (0.02 International units per cc.). Experiments are now in progress with alcoholic extracts of human cerebrospinal fluid which are designed to indicate if there is in human C. S. F. any pituitary-like oxytocic material in the much smaller concentrations postulated by workers other than Dixon.

Reactions of pial vessels. F. E. FRANKE and L. D. SEAGER with the assistance of R. A. Smith.

The pial vessels of the dog (arteries and veins of various sizes) have been observed through a window in the skull, the window having a spheroid form to prevent flattening of the brain surface. Continuous micrometric

measurements of the vessels; respiration, arterial, cerebrospinal fluid, femoral venous and torcular pressures were recorded simultaneously on the kymograph when subjecting the animal to the following procedures: 1, intravenous, intracarotid and local administration of adrenalin and sodium carbonate; 2, inhalation of amyl nitrite; 3, occlusion of carotids alone and of carotids and vertebrals and of venous return from the brain; 4, intracranial pressure changes.

The changes in vessel size are determined by the balance between direct effects on vessel tonus, indirect effects from their innervation and the mechanical effects of intra-vascular and intra-cranial pressures. Veins and arteries may constrict or dilate in the same or opposite direction depending on the forces operative at the moment. Variations in the vessel tonus frequently overcome effects from changes in intra-vascular and intra-cranial pressures. The opposite is also frequently observed.

Bilateral representation of the lower extremity in the motor cortex of the chimpanzee. J. F. FULTON.

In the chimpanzee ablation of the motor representation of the lower extremity from one cerebral hemisphere produced in the opposite hind extremity all the classical symptoms of monoplegia which accompany the corresponding lesions in man. During the first 24 to 48 hours after the extirpation all reflexes were abolished in the affected extremity, but the sign of Babinski and other pathological plantar reflexes return early,¹ being followed by the knee-jerks and ankle-jerks. There was little disturbance in the ipsilateral hind extremity apart from a slight abnormal tendency toward lateral deviation of the toes during voluntary movement, which persisted for three or four days. There had been, however, no clear-cut response of the ipsilateral extremity on faradic stimulation of the motor area prior to extirpation.

When the corresponding motor representation of the *second* hemisphere was removed, *e.g.*, after an interval of one to two months, the newly paralyzed limb passed into a state of profound depression of reflex activity lasting for three to seven days in the three chimpanzees studied, *i.e.*, for approximately twice the interval required for reappearance of reflexes of the first extremity after the primary lesion. Furthermore, the extremity *ipsilateral* to the second lesion also suffered depression of reflexes, and there was unmistakable diminution of motor power together with a permanent alteration of the posture of the toes. Moreover, on eliciting the sign of Babinski in this extremity very strong lateral deviation of the toes occurred, which had not been seen after removal of the contralateral leg area alone. Several months after the second ablation the reflexes of the two hind extremities became similar, with permanent postural disturbance and marked motor deficit affecting chiefly the more distal joints, *i.e.*, ankle and phalangeal.

These observations give functional significance to the uncrossed pyramidal pathways described in the chimpanzee by Leyton and Sherrington,² the presence of which we have been able to confirm. The work has also thrown light on the symptoms of cerebral diplegia in man.

¹ Fulton, J. F. and A. D. Keller. The sign of Babinski: a study of the evolution of cortical dominance in primates. Thomas, 1932.

² Leyton, A. S. F., and C. S. Sherrington. Quart. Journ. Exper. Physiol., 1917, xi, 135.

The digestive leucocytosis question. W. E. GARREY and VIRGINIA BUTLER.

The evidence for the existence of a digestive leucocytosis is far from conclusive. We have therefore made experimental tests which aim to eliminate other conditions which cause variations in the leucocyte count. Subjects were placed in the recumbent posture for one hour with a resulting drop in the leucocyte count to its lowest (basal) level, then fed a large meal of protein; in other instances a carbohydrate meal was administered. Fluid was withheld to avoid the effects of gastric distention. The leucocyte count remained unchanged; the basal level stayed constant for four hours or more, provided the recumbent posture was maintained and other disturbing factors were avoided. In a second series of tests it was found that the basal leucocytic level reached in any individual case was independent of the morning or noon-day meal. Sudden distention of the stomach by air, water, or other fluids caused a definite leucocytosis which subsided to the basal level in one-half hour. Sudden changes in gastric temperature by either hot or cold water had a similar transient effect. Since the effects did not persist and were induced without the intervention of digestion the conclusion is warranted that there is no leucocytosis consequent upon the absorption of the products of digestion. Experiments upon dogs under barbitol anesthesia confirmed the results and conclusions drawn from the data on man. It appears that all variations in the leucocyte count due to a meal are the consequence of sudden vascular changes and are the result of the activities of the individual incident to getting the meal in greater measure than to the changes induced by the food or drink partaken.

The end of the spike-potential of nerve and its relation to the beginning of the after-potential. HERBERT S. GASSER and HELEN TREDWAY GRAHAM.

A determination was made of the form of the spike of the action potential of frog sciatic nerve by a technique differing from the ones previously used. The essential features of the procedure were the avoidance of diphasicity by treating the end of the nerve with potassium chloride after it had been killed by heating, and the reduction of the after-potential to a minimum by recording one of the first responses after preparation and by keeping the nerve cool (14° as compared with 24° at which the frogs were kept).

The form of the monophasic spike as it would occur at laboratory temperature was then calculated by dividing the times of all parts of the curve by the factor which would make the crest time 0.3σ . From this curve was derived the theoretical form of the partially diphasic spike that would be obtained when the lead-off electrode on the killed end of the nerve picks up a small wave. For the range of conditions that would occur in an experiment all such reconstructions showed that the last part of the first phase outbalances the second so that a negative wave exists after the apparent portion of the diphasic artifact. This second negative wave of the axon spike potential was identified experimentally in the various combinations in which it occurs with the after-potential. Records showing these combinations are demonstrated. When the end of the spike is thus identified it is possible to evaluate the form of the beginning of the after-potential. The after-potential starts before the spike ends and increases in magnitude for a period dependent on the condition of the nerve.

Action potentials of single muscle fibers. S. GELFAN and G. H. BISHOP.

As Gelfan has shown, a single muscle fiber can be stimulated electrically

with micro-electrodes to contract without conduction of the impulse along the full length of the fiber. We have investigated the question whether such a shortening was accompanied by a normal action potential.

We first recorded diphasically the normally conducted impulses in the frog's sartorius, in single fibers, using micro-electrodes, and found them to be exactly like those recorded previously by Bishop and Gilson using fine wire electrodes, each phase being "triphasic" as Craib has described for tissues immersed in solutions. Then, since in the frog's retrolingual membrane where single fibers are isolated, these fibers are at most 3 mm. long, we brought the electrodes to within 3 mm. separation on a fiber of the sartorius, and so arranged the electrodes in a bridge circuit that the shock artefact was at a minimum, and that the two phases of the diphasic record could be detected even when they were partially superposed at this distance of conduction. Records with the same arrangement of apparatus from the retrolingual fibers were practically identical with records from the sartorius, when the impulse was conducted.

With a stimulus intensity that was sufficient to cause only a portion of the single fiber to respond, no action potential whatever could be recorded accompanying the response. In two preparations, presumably depressed, even strong stimuli failed to give conducted impulses. In these preparations, the shortening of the muscle lasted as long as the duration of the current. Now with short durations of current, these responses, which must obviously have been galvanic contractures, could not, by visual inspection under the microscope, easily be distinguished from non-conducted responses to shocks. It is thus conceivable that the submaximal responses of the single fiber may be of the nature of brief contractures. Such a viewpoint would obviate any fundamental difference between contraction and contracture, except for the absence of the action potential in the latter.

The validity of the Hofmeister series in heart and striated muscles. E. GELLHORN.

The influence of different anions (Na-salts) on the automaticity in heart (*Rana esculenta*) is demonstrated with Straub's preparation and a heart strip. When a standstill is obtained through a non-electrolyte (cane sugar) automaticity may be restored by a solution containing 30 per cent isotonic Na-salt + 70 per cent isotonic non-electrolyte solution. The efficiency of different sodium salts follows the Hofmeister series, citrate being the least effective and SCN the most effective anion. The latter one is many times more effective than Cl.

Schwarz showed that under these conditions the validity of the Hofmeister series can be shown in striated muscle. Our experiments, however, show that the use of non-electrolytes is not essential. Nerve-muscle preparations of *Rana esculenta* were stimulated indirectly through condenser discharges in solutions consisting of 30 per cent isotonic Na-salt solution and 70 per cent Ringer's solution. If the muscle ceases to react the solution is replaced by another one containing a Na-salt with a different anion. Thus the great difference between various anions, and particularly the superiority of SCN is shown. Since all solutions are of the same pH and great differences are observed between Cl and SCN, the physiological specificity of anion effects at a constant pH is evident even when these are monovalent.

The respiratory quotient of working isolated muscles with low carbohydrate content. C. L. GEMMILL.

The respiratory quotient was determined for the frog's sartorius muscle contracting four times a minute. In normal muscles with an average total carbohydrate content of 0.85 per cent (glucose) the respiratory quotient varied from 0.93 to 1.04 with an average of 0.98. Seven muscles with their carbohydrate content lowered by means of insulin convulsions gave quotients varying from 0.91 to 1.01 with an average quotient of 0.95. In this series the lowest carbohydrate content obtained was 0.15 per cent. The accuracy of the method is not sufficient to determine the percentage of carbohydrate and protein used as protein oxidation in muscles has a quotient of 0.95. It does indicate, however, that fat is not burnt as such for the energy of contraction under these conditions.

Nerve conduction velocity and equilibration. R. W. GERARD and W. H. MARSHALL.

Though a medullated nerve cannot ordinarily be fatigued to complete inactivity, it does undergo changes as a result of intense activity. Previous work has demonstrated decreased energy liberation and chemical change per impulse, altered action potentials, heightened threshold and prolonged refractory period in nerve after a period of rapid tetanization as compared to the resting state. The amount of change from the norm increases with frequency and duration of excitation until some equilibrium level, characteristic for the given conditions, is attained, when the nerve is in a state of equilibration.

It was to be anticipated that conduction velocity would be similarly decreased in such equilibrated nerve. This has been measured by leading conducted action potentials, after amplification, to a cathode ray oscillograph. The distance between stimulus artifact and start of the action potential on the tube, after calibration with a known time curve, can be expressed as conduction time with an accuracy greater than 0.05%. Possible sources of error, such as local effects under the tetanizing electrodes, conduction slowing during a prolonged refractory period, current spread, etc., were carefully eliminated.

Over a dozen complete experiments on bullfrog sciatics and dog phrenics (at 21°) have without exception demonstrated a decreased conduction velocity following a 10 to 15 minute tetanus with 300 to 400 maximal shocks per second. Velocities as low as 60 per cent normal have been obtained a few seconds after terminating the long tetanus. At one minute, the rate had risen, on the average, to 85 per cent, at 3 minutes to 90 per cent normal. Changes often were still progressing 15 minutes later, though a period of supernormal velocity was sometimes passed through before this time.

We conclude that conduction velocity, like many other attributes of nerve activity, is decreased during the equilibration associated with activity and, also like the others, returns gradually over a 10 to 15 minute period of rest to its original value.

Spontaneous changes in type of breathing and changes occurring in respiratory movements under varying mechanical conditions. ROBERT GESELL and CARL A. MOYER.

The nervous and chemical regulations of respiration are being studied by recording pulmonary ventilation, intra-tracheal pressure and changes

in costal and abdominal circumferences at six levels. The object is to emphasize the interplay of reflex and chemical factors and the peripheral and central coordination accompanying respiratory movements under varying conditions.

The degree of costal and abdominal breathing varied enormously in different animals. Breathing changed spontaneously from one type to another in the same animal. In one experiment a repeated alternation between the costal and abdominal type occurred. The type of breathing influenced the response to mechanical conditions.

Simple mechanical asphyxia produced by closure of the tracheal cannula at the end of inspiration or expiration commonly led to immediate fixation of the chest in the inspiratory or expiratory position with little or no costal excursions. Abdominal excursion continued. In animals in which costal respirations are highly developed, fixation and excursions may be reversed.

During inspiratory and expiratory mechanical asphyxia when expiration or inspiration only is possible costal excursions were mostly missing but abdominal excursions continued. The expiratory lung volume may or may not change. The change in lung volume may be reflected equally or differently in the costal and abdominal segments.

Phrenectomy abolished costal fixation in all types of asphyxia.

Vagotomy modified the asphyxias. Two effects were reducing the rhythm changes and costal fixation.

Increased intra-tracheal pressure led to a differential expansion in the costal and abdominal segments varying with the animal. Expiration is little affected in the chest, but markedly prolonged in the abdomen. The greater the pressure, the greater was the inhibition. Increased intra-tracheal pressure produced a greater increase in lung volume when applied during inspiration. The inhibition produced, however, was little greater.

Pneumothorax with the lungs collapsed and chest open led to variable expansion of all segments. There was usually a marked increase in rate. The increase in rate as a rule was very much greater if the chest was closed. Vagotomy and phrenectomy greatly modified the response to pneumothorax.

Changes in respiratory movements occurring as a result of nerve section and stimulation of sensory nerves. ROBERT GESELL and CARL A. MOYER.

The development of costal respiration following phrenectomy varied considerably. In some experiments with predominant abdominal respiration, phrenectomy led to respiratory and circulatory failure. Temporary mechanical asphyxia accelerated the development of costal respiration.

The increase in depth of respiration following vagotomy was evenly distributed in the chest and abdomen. Respiration was changed in only two out of forty experiments from costal to abdominal. Differing from the rabbit, there was very little or no change in the expiratory circumferences. Dorsal root extraction (1-9 thoracic alone or with lower 5 cervicals) seldom had obvious effects on basal ventilation. In one experiment they were fatal. Combined with vagotomy and phrenectomy they were more frequently fatal. In some animals they are well tolerated. There were no obvious changes in type of respiration or in response to mechanical asphyxias, but mechanical asphyxias sometimes caused respiratory failure.

When extraction of the dorsal roots modified ventilation by excitation

the effects were unevenly distributed depending on the location of the nerve and the sequence of extraction. Stimulation of the central ends of the cervical, saphenous, vagus and superior laryngeal nerves produced striking segmental differences in response. In one experiment, saphenous stimulation elicited twice as many costal as abdominal movements. Stimulation of the vagus nerve produced many types of response: inhibition, acceleration, inhibition followed by acceleration, an initial inspiration followed by an early expiration and a pause, a marked increase in the inspiration in progress. The rate and extent of expiration showed marked segmental variation. Rapid intermittent stimulation, properly placed, led to rapid shallow respiration timed to stimulation. This finding appears significant in the theory of respiratory rhythm. Stimulation of the superior laryngeal produced a prolonged inhibition accompanied by a large decrease in lung volume sometimes reflected to a greater extent by the costal segments.

Changes in respiratory movements accompanying chemical administrations.

ROBERT GESELL and CARL A. MOYER.

The common effect of carbon dioxide was an increase in costal and abdominal excursions resulting from a combined decreased expiratory and increased inspiratory circumference. The common effect of lowered alveolar oxygen was an increase in costal and abdominal excursions associated with an increase in both expiratory and inspiratory circumferences. In some experiments increased alveolar carbon dioxide and decreased alveolar oxygen produced different effects in the costal and abdominal segments. Cyanide and sulphide produced effects resembling those of lowered alveolar oxygen. Sodium bicarbonate produced effects resembling those of carbon dioxide. In general sodium carbonate elicited reverse effects.

When stimulation of the central end of the vagus produced inhibition this was abolished or reduced by carbon dioxide administration. When stimulation produced acceleration this was also diminished. Carbon dioxide invariably diminished the inhibitory effects of increased intra-tracheal pressure by agreeing with the results of Hammouda and Wilson on increased lung volume. In contrast are the effects of lowered alveolar oxygen, which sometimes may be smaller or missing or which may increase the inhibitory pause produced by vagal excitation. In two experiments in which vagal excitation produced respiratory acceleration the effect was still greater augmentation during stimulation plus lowered alveolar oxygen. From the results of several experiments the inhibitory effects of stimulation of the superior laryngeal appear to be diminished by carbon dioxide and variable with lowered alveolar oxygen just as is vagal inhibition.

The similarity of results obtained by Glazer, by Winkler and by Gay on the effects of carbon dioxide and low oxygen on spinal reflexes offer support to our present findings which appear significant for explaining differences in rate and amplitude of ventilation occurring during lowered alveolar oxygen and increased alveolar carbon dioxide.

Latent tolerance in diabetes mellitus. R. B. GIBSON.

Continuing previously reported observations (1928), a number of mild diabetics have been restored promptly to a normal carbohydrate tolerance when a Woodyatt type diet on which they were controlled without insulin

was changed to a diet of protein 46 grams, carbohydrate 204 grams or more, and fat 100 grams with insulin in progressively decreasing dosage. More severe cases show a partial response; they reach a normal tolerance but do not maintain this, or they improve as indicated by prompt insulin reduction when returned to routine management and even are able to dispense with insulin. Certain patients, however, do not respond to this procedure. One patient who has been routinely managed by diet and later both diet and insulin recently developed repeated autogenous hypoglycemic attacks following two separated periods of tolerance stimulation (each time after recovery from diabetic coma) with subsequent stabilization of the blood sugar within normal limits on a general diet. These results confirm the idea earlier expressed that a dormant rather than a functionally inadequate mechanism may obtain in diabetes and that it may be stimulated to activity under appropriate conditions.

A study of the osmotic relations of blood and gastric juice. A. GILMAN and GEORGE R. COWGILL.

Using the Hill vapor pressure method, 96 simultaneous observations were made on the osmotic pressure of blood and gastric juice obtained from five Pavlov pouch dogs. The electrolyte concentration of the blood was widely changed by oral administration of large quantities of either hypertonic saline solution or water. Expressing osmotic pressure in terms of milli-equivalents (m.eq.) of an osmotically equivalent salt solution, the variations in the osmotic pressure of the blood produced by this procedure ranged from 120 to 178 m.eq. During the course of the experiment, while the blood was exhibiting such marked changes, gastric juice was obtained under histamine stimulation. The results show that with an increase or decrease in the osmotic pressure of the blood there is a parallel change in the osmotic pressure of the gastric juice. In the experiments in which salt was administered the average difference between the respective o.p. of the two fluids was equal to an o.p. of 3.7 m.eq. NaCl, the blood being hypertonic. On the other hand, following water administration the average difference was 4.74 m.eq., the gastric juice being hypertonic. There was also observed an inverse relationship between the o.p. of the blood and the rate of secretion of gastric juice. The more dilute the blood, the more rapid was the flow of juice in response to a definite stimulus. Chemical analysis of the chloride content of the gastric juice showed that the total o.p. of this fluid may be attributed to this anion in conjunction with any cation with which it may be associated.

Integration of nerve impulse effects. A. S. GILSON, JR.

A single effective shock applied to the vagus nerve of the turtle may produce depression in the strength of the atrial beat in the heart. The depression does not appear immediately in maximal degree but develops to a maximum during a period extending through several cardiac cycles. There then follows a period of progressive recovery. Curves showing the amount of depression plotted against time resemble skew distribution curves in general form. They may, however, be satisfactorily fitted by curves of an equation expressing the change in concentration of a substance under the effects of both diffusion and chemical destruction.

A study of such depression curves showed that an increase in strength of a single shock to the nerve produced an increased maximum degree of atrial depression between the limits of a just visible threshold and the

maximal depression produced by a single shock. Such an increase of shock strength did not, however, alter the time course of the curve. Two (or more) stimuli to the vagus produced summation of effect in such a manner as might be predicted from a knowledge of the effects of a single shock and of the temporal dispersion of the two (or more) effects.

Experiments show clearly that, in the preparation studied, the degree of nerve impulse summation observed depends essentially upon 1, the time-intensity course of the effect of a single unit fiber impulse, and 2, the number of nerve fiber impulses activated within a given period of time. It is believed that this same argument holds for any nerve or group of nerves producing increased effects by iterative activity.

A study of the rôle of inhibition in color contrast. C. H. GRAHAM.

It has been possible to study the retinal interaction in response to various wave-lengths by using the fusion frequency of a flickering light as the index of excitation. Under these conditions it is found that two flickering semi-circles of *the same* spectral distribution and at the same intensity of illumination have a higher fusion frequency than one alone. The retinal behavior underlying this response is interpreted as that due to summation of excitation over lateral pathways in a manner similar to that discussed by Granit.

The results are different when the two flickering patches are of different spectral distributions. When, for example, a red semi-circle and a blue semi-circle, originally at the same fusion frequencies, are viewed flickering synchronously and adjacent to each other, the fusion frequency of the total patch (red and blue) is *no higher* than the fusion point of either alone. (These experiments have been performed with various combinations of wave-lengths and in some cases we have found that one area decreases the fusion frequency of the adjacent patch below that which it has when present alone.) Since either will "sum" with an adjacent patch of similar spectral distribution and fusion frequency, the results with the dissimilar patches are interpreted as being due to mutual inhibition.

That the picture of interaction thus presented describes the phenomena of color contrast may be seen from the following example. A red area adjacent to a white inhibits the red component of the white since the excitatory processes of the red components of the adjacent areas are at different levels. Reciprocally, the blue and green of the white inhibit the feebly acting blue and green functions in the red area. Thus, the red appears with greater saturation than when alone and the white area assumes a greenish blue tinge.

The peripheral innervation of the heart as revealed by the specific action of the myoneural drugs on isolated strips of the turtle's heart. CHARLES W. GREENE and KARL E. MANEVAL.

Evidence from the older literature supporting the view that the extrinsic innervation of the heart is by fibers more or less equally distributed to all chambers has been slowly displaced since the discovery of the nodal system in the heart of mammals and the higher vertebrates by evidence that the extrinsic nerve control is through regulation of the special pace-making tissues. We have examined different local regions of the heart of the turtle by the isolated strip method, using the reaction to drugs specific for myoneural tissues as an index to the type and degree of nerve distribution to the local region.

The behavior of three critical cardiac regions is presented: 1, the sinus and adjacent basal tissues; 2, the apex of the auricle, which is free of ganglionic cells, and 3, the ventricular apex, the reactions of which have long been assumed but not clearly proven to be purely myocardial in type. On these preparations physostigmine sulphate has been used to stimulate and atropine sulphate to paralyze inhibitory vagal fibers, and adrenalin to stimulate thoracic sympathetic or augmentor terminal cardiac endings. The reactions are consistent and are categorically stated as follows:

1. Physostigmine inhibits the automatic rhythm of the sinus, but does not in any way affect the tone waves. Auricular apex strips do not often exhibit rhythm but when stimulated to rhythm are also inhibited by physostigmine, but the tone contractions are not affected. The ventricular apex apparently is not affected by physostigmine in any of its activity.

2. Atropine has no influence on the normal rhythm or tone of either of the three preparations. But it promptly restores rhythm in sinus or auricular strips that have been inhibited by physostigmine. After an atropine release inhibition can not be repeated.

3. Adrenalin stimulates acceleration in the rhythm and augmentation of amplitude of sinus and of auricular apex strips. It also promptly inhibits all tone waves, an unexpected reaction in view of the failure of atropine and physostigmine to produce any change in tone. Adrenalin on the most sensitive strips reacts in dilutions of one part in one billion.

On the ventricular strips very much stronger solutions of adrenalin, one to one-hundred-thousand, induce only appreciable augmentation of amplitude, either indicating very few accelerator endings in the ventricles, or compelling one to subscribe to the direct action theory.

Since no conclusive evidence of direct muscular effects of these drugs have appeared, the following conclusions are drawn.

1. Terminal nerve endings of the isolated heart strips are sensitive to specific myoneural drugs.

2. The tone producing mechanism receives no motor nerves. But it is richly supplied with inhibitory fibers via the thoracic sympathetics.

3. There is an evident cardiac gradient of nerve distribution in the turtle in both inhibitory and motor nerves. The sinus is richly supplied by both, while the ventricle receives a few accelerator fibers and no inhibitory fibers, except in a rare individual animal.

The physiological mechanism of thirst. MAGNUS GREGERSEN.

The amount of water taken by dogs during 1 or 2 hours of panting is increased after extirpation of the salivary glands. A deficient salivary flow may, therefore, even in the absence of bodily dehydration, cause thirst and increased water intake.

Graphic records of the drinking of dogs reveal that water is taken almost solely during 2 to 3 hours after feeding, regardless of the time of feeding. If the giving of water is delayed for 5 to 6 hours after feeding, the 24-hour intake is reduced to $\frac{1}{2}$ or $\frac{1}{4}$ of that obtained with water *ad libitum* throughout the postprandial period. Postprandial need for water is therefore in part only temporary. When no water is given during the meal or after, the plasma volume (vital red method) suffers a temporary drop of 20 to 30 per cent, and may return to normal without further intake of water. Parallel changes occur in the salivary flow (polypnea secretion). Two days' water deprivation produces no greater fall in salivary flow and plasma

volume than does the normal meal which temporarily withdraws fluid from the blood in the form of digestive juices.

These observations, coupled with the well-known dependence of the salivary flow upon blood flow through the glands and the intimate relation existing between the latter and total blood volume, suggest that the dry mouth in dehydration arises from circulatory adjustments following upon a decrease in plasma volume.

The calorigenic action of lecithin. DONALD E. GREGG.

The possibility that lecithin is an intermediary in the metabolism of fat was studied on 3 dogs by determining the changes in heat production, in the respiratory quotient and in the urinary nitrogen after feeding 60 to 90 grams of lecithin and after injecting it intravenously (in salt solution) in amounts ranging from 0.3 to 2 grams per kilo.

In addition to control of the apparatus through alcohol checks and of the animals through repeated determinations of their basal metabolism, the effect of saline injections *per se* was taken into account when lecithin emulsions were administered intravenously.

The results show that some animals respond to intravenous injections of saline by increased nitrogen excretion (+22 per cent) and a slight augmentation of heat production (+6.5 per cent) which cannot be overlooked in evaluating the effects of lecithin injections.

Lecithin given *per os* has a specific dynamic effect much less than an isocaloric amount of neutral fat, does not affect the respiratory quotient but does increase the urinary nitrogen excretion. When given intravenously in amounts up to 1 gram per kilo it has no calorigenic effect whatsoever, the non-protein R.Q. gives no indication that lecithin is oxidized and the nitrogen excretion also shows no greater deviations than those following saline injections alone.

These metabolic studies therefore offer but little support for the view that lecithin is concerned in the intermediary metabolism of fat or that it is a readily available source of material for oxidation.

Blood chemistry variations in fifty women and fifty men. ESTHER M. GREISHEIMER and FRED P. ARNY.

The subjects were faculty members, students, and parents of students. All were in good health. The blood was drawn by venepuncture between seven and eight o'clock in the morning, after a twelve to fourteen hour fast.

TABLE 1
Means (mgm. in each 100 cc.)

	WOMEN	MEN	ENTIRE GROUP
Sugar.....	83.28 \pm 0.71	84.42 \pm 0.71	83.85 \pm 0.50
Calcium.....	10.673 \pm 0.046	10.506 \pm 0.027	10.590 \pm 0.027
Inorganic phosphate.....	3.462 \pm 0.044	3.402 \pm 0.056	3.432 \pm 0.030
Sodium chloride.....	585.0 \pm 1.2	579.1 \pm 1.2	582.0 \pm 0.89
Urea nitrogen.....	13.77 \pm 0.25	13.22 \pm 0.26	13.49 \pm 0.18
Total non-protein nitrogen.....	32.54 \pm 0.37	35.06 \pm 0.33	33.80 \pm 0.26
Uric acid.....	3.112 \pm 0.037	3.358 \pm 0.053	3.235 \pm 0.033

The following constituents were determined by the methods designated: sugar, improved Shaffer-Somogyi; serum calcium, Clark-Collip modifica-

tion of the Kramer-Tisdall; inorganic phosphate, Fiske-Subbarow; serum chloride, Whitehorn; urea, direct nesslerization of unlaked blood filtrate (after digestion of urea of whole blood by urease paper); total non-protein nitrogen, Koch-McMeekin oxidation and direct nesslerization of tungstate-sulphuric acid filtrate; and uric acid, Benedict's method.

The means are presented in table 1, and the standard deviations are presented in table 2. The results for each sex and for the entire group are given.

TABLE 2
Standard deviations

	WOMEN	MEN	ENTIRE GROUP
Sugar.....	7.42 \pm 0.50	7.45 \pm 0.50	7.45 \pm 0.35
Calcium.....	0.481 \pm 0.032	0.285 \pm 0.019	0.399 \pm 0.019
Inorganic phosphate.....	0.462 \pm 0.031	0.458 \pm 0.031	0.453 \pm 0.022
Sodium chloride.....	12.66 \pm 0.85	13.01 \pm 0.88	13.16 \pm 0.63
Urea nitrogen.....	2.63 \pm 0.18	2.74 \pm 0.18	2.70 \pm 0.13
Total non-protein nitrogen.....	3.84 \pm 0.26	3.49 \pm 0.23	3.88 \pm 0.18
Uric acid.....	0.386 \pm 0.026	0.553 \pm 0.037	0.493 \pm 0.023

Observations on the hormone of the adrenal cortex. ARTHUR GROLLMAN and W. M. FIROR.

Swingle and Pffiffer's method of preparation of the cortical hormone of the adrenal may be appreciably shortened and improved by extracting the glands with acetone, instead of alcohol. After removing the solvent and extracting with benzene, epinephrine is removed by dilute alkali. This simplified process yields a product free of the toxin which is often present in the extracts prepared by Swingle and Pffiffer's or by Hartman's method. This toxin is an indole-phenolic derivative probably identical with the "omega" substance formed on exposing epinephrine to sunlight. It is cherry-red in color turning brownish-black on standing and may be separated by extracting the active principle with ether. The use of colored extracts is to be avoided particularly in clinical cases. Suspected solutions may be tested by injecting one cubic centimeter into a mouse, death ensuing in a few minutes to an hour depending on the amount of the toxin present.

The most satisfactory test animal for determining the potency of the extract is the rat. Removal of the veil of connective tissue surrounding the adrenal bodies and avoidance of crushing of the glands resulted, in our series of over 100 animals, in death usually within 1 to 12 days after operation or cessation of the extract.

The pre-operative treatment of animals is an important factor in determining their survival period after adrenalectomy. Cats subjected to cold for several days before operation show the cardinal signs of adrenal insufficiency (loss of weight, tendency to infection, and lack of resistance to cold) and may die (particularly young animals) even after unilateral adrenalectomy. This importance of pre-operative treatment accounts for the frequently observed apparent greater prolongation of life after two-stage operations as compared to the one-stage removal. We have found that the average amount of extract necessary to keep animals in good condition after adrenalectomy is greater than that claimed in the literature.

Excitability of the single-fiber nerve-muscle complex. H. GRUNDFEST.

The voltage capacity relation has been studied in single nerve and muscle fibers of the frog retrolingual membrane, in order to test the validity of Lapicque's "law of isochronism." The preparation has been made according to the method of Pratt. The relatively large capillary electrodes ($20-80\mu$) insure all-or-nothing responses in the muscle fibers. A 10,000 ohm shunt is placed across the electrodes, so that 1μ is approximately equivalent to a duration of 4σ .

The chronaxies of the nerve fibers vary from $0.02-0.1\sigma$ and those of the muscle fibers from $0.1-0.4\sigma$. The ratios of the nerve and muscle chronaxies range from 1:2 to 1:10. Furthermore, curarization does not affect the chronaxies of muscle fibers. Because of these results, it is concluded that the "law of isochronism" is not valid for the retrolingual preparation.

The heterochronism in the retrolingual complex further manifests itself by compound voltage-capacity curves of the type described by Lucas and Rushton. It has been possible, in these experiments, to demonstrate that their compound nature is due to the different excitabilities of muscle and nerve fibers and to secure the suppressed branches of each component curve.

The effect of electrode size upon chronaxie of nerve and muscle fibers has been studied. The chronaxies of both nerve and muscle are increased with increase in electrode area, but the effect is greater with muscle. It is therefore possible to homologize the compound curves obtained in the present work with those of Lucas and of Rushton which have different time relations because of the very large electrodes used by these observers.

The chronaxie of a single nerve fiber also varies with the amount of tissue surrounding the fiber.

A differentiation stimulus exerted by some amino acids during development.

F. GUDERNATSCH and O. HOFFMAN.

In a study of the specific effects of various α amino acids on development of *Rana sylvatica*, indications were seen of the presence in some acids of a factor for differentiation. This observation was made only on acids which are structurally related to diiodo-tyrosine, viz.: phenylalanine, tryptophane and tyrosine. Amino acids, in various combinations of two, provided the only nitrogen source in the food. In the controls with a basal diet of carbohydrates, fats and cod liver oil, 1 animal out of 40 produced semblance of hind limb buds. In the acid fed groups, hind limb buds were produced by a few animals: in 2 groups out of 12 glycine groups; 1 out of 10 arginine groups; 2 out of 11 cystine groups. On the other hand, advanced differentiation was seen in 50 per cent of the phenylalanine and in 65 per cent of the tyrosine cultures. In the tryptophane cultures, the percentage likewise was high and the extent of differentiation very great. In fact, in one of these groups, which had been treated with cystine + tryptophane for twelve weeks, one animal practically completed metamorphosis. Even in cultures which were definitely negative as far as differentiation was concerned, there were signs of differentiation when phenylalanine, tyrosine or tryptophane was added to the mixture.

After 14 weeks, acid fed animals which exhibited no signs of differentiation were treated with diiodo-tyrosine. The response to the treatment was characteristic. It was slow in the groups which per se produced no differentiation (alanine, leucine, arginine, cystine, etc.), while the diiodo-

tyrosine reaction occurred almost immediately in the phenylalanine, more markedly in the tyrosine and still more so in the tryptophane groups. Any of the three latter acids combined with diiodo-tyrosine acted almost as quickly as twice the concentration of diiodo-tyrosine alone. It is possible then that phenylalanine, tyrosine and tryptophane (perhaps also histidine) have differentiation potencies related to those of diiodo-tyrosine.

The respiratory quotient as an index of meal intervals. HOWARD W. HAGGARD and LEON A. GREENBERG.

A method has been developed for determining the respiratory quotient without the coöperation of the subject. It has been applied to more than one hundred individuals ranging in ages from infants to the aged. In each case the quotient was determined at hourly intervals throughout the day. In selected cases the blood sugar concentration was determined simultaneously. From the findings it is believed that the proper meal interval can be determined for each age.

Inhibition of lactic acid formation in brain and kidney produced by intravenous injection of sodium monoiodoacetate. JOHN HALDI.

A solution of monoiodoacetic acid neutralized with sodium carbonate was injected into the femoral vein of dogs. In eight experiments small dogs weighing approximately 4 kilos were injected with 42.8 to 126.0 mgm. monoiodoacetic acid per kilo body weight. The animal was guillotined, the brain and kidney quickly removed and one kidney and a portion of one half of the brain frozen immediately in liquid air. The other kidney and a corresponding portion of the other half of the brain were incubated for 10 minutes and then frozen in liquid air. In no instance was there any increase in lactic acid in the incubated kidney. Incubated brain tissue showed practically no increase in four experiments whereas in an equal number of experiments the increase varied from 53.4 to 95.2 mgm. per cent. In nine control experiments on dogs anesthetized with morphine and urethane—the injected animals were anesthetized in this manner—the average lactic acid increase in kidney and brain tissue incubated 10 minutes was 36.3 and 113.3 mgm. per cent respectively.

A characteristic rate of lactic acid formation has been reported for excised brain, kidney, muscle and testicle. The suggestion was offered that these differences might be due to different concentrations in the various tissues of enzymes controlling lactic acid formation. The present experiments are interpreted as evidence in support of this supposition.

*A chick heart method of biological assay—I. Digitalis.*¹ EDITH M. HALL.

In growth studies done several years ago, the chick embryo was used as experimental material. It was thought that this same animal could be used for the assay of digitalis. Accordingly, a technique was worked out whereby, with a sufficient number of eggs under standard conditions, the potency of digitalis as contained in tinctures and other preparations, can be determined. The method is quite comparable in accuracy to those in standard use, namely, the guinea pig and the frog methods. The advantage lies in simplicity, availability of material and perhaps more

¹ This method will be demonstrated at the Federation Meetings.

accurate checking of results. Full details of the method with comparisons are to be published in the American Journal of Pharmacy.

Influence of sulphhydryl and sulfoxide on aberrant disorganized growths in the regenerating chela of the Hermit crab. FREDERICK S. HAMMETT and DOROTHY WALL HAMMETT.

Aberrant growths arise from developmental defects, junctional tear injuries, and the most anterior center of proliferation in the regenerating right chela of the Hermit crab. The basis of formation of growths from the developmental defects and the tears is the high intensity of proliferation natural to early regenerative growth combined with a delay in expression of organization capacity which arises from its dislocation through injury. These growths are accelerated by sulphhydryl and retarded by its partially oxidized derivative sulfoxide. The growths which emerge from the tip end of the chelae only occur when cell proliferation has been accelerated by sulphhydryl. They are never found in control or sulfoxide exposed chelae. They are due to the combination of a naturally occurring proliferation center of high intensity and the experimental exposure to the natural stimulus to growth by increase in cell number. They are true disorganized overgrowths induced by the SH group.

The effect of stimulation of the cervical sympathetic trunk upon the respiratory metabolism of rabbits. H. F. HANEY.

This investigation is concerned with a study of the rate of respiratory metabolism following stimulation of the cervical sympathetic trunk. A modified Haldane open circuit apparatus was used in all of the metabolism tests. In order to establish individual normal rates, each of the 30 rabbits used thus far have had 2 to 5 metabolism tests before beginning an experiment. The animals were taken off feed 15 to 20 hours before beginning each test.

In series 1, the sympathetic trunk was cut low in the neck and the animals allowed to recover. In series 2, the trunk was cut, just as in series 1, and immediately following, interrupted tetanic stimulation was applied to the peripheral end over a period of 1 to 3 hours. Most of the operations were carried out with the use of very light chloral hydrate-urethane anesthesia. A few animals were given ether during the operation, and the stimulation carried out without anesthetic. Metabolism tests were performed in some cases daily and in others every other day for the first 10 days after the operation, and following this, every 2 to 5 days over a period of 40 to 80 days.

Those animals in series 1, in which the trunk was cut but not stimulated, showed an average maximal variation above the normal of 14 per cent. The highest single rate recorded in this series was 23 per cent above normal. Four of the 6 rabbits used in this control group did not vary more than 15 per cent above the normal figure.

The average maximal variation above the normal rate of metabolism in series 2 in which the peripheral end of the cut nerve was stimulated, was 37 per cent. Twenty of the 24 animals used in this series showed a rise of 20 per cent or over, 15 a rise of 30 per cent or over, 9 a rise of 40 per cent or over, 6 a rise of 50 per cent or over, and 5 a rise of 60 per cent or over. Typically, the rate of metabolism began rising at the second day and rose to a maximum which, in 17 of the 24 animals, occurred between the second

and eighth days following stimulation. The return to normal occurred usually between the twentieth and fortieth days following stimulation, but this is subject to considerable variation. The results are most naturally interpreted as being in favor of increased liberation of thyroid hormone.

The effect of dark adaptation on the discharge of impulses in single optic nerve fibers. H. K. HARTLINE.

A method has previously been described for recording impulses in single optic nerve fibers from the eye of *Limulus polyphemus*. The frequency of discharge of nerve impulses in these single photosensory units was found to be higher the brighter the stimulating light. Another factor which affects the discharge is the degree of dark adaptation of the eye; it is with this that the present paper is concerned.

Following illumination of the eye by a bright light for about 5 minutes, the discharge of impulses in response to a stimulating light of given intensity was studied. Two features are especially noteworthy:

1. Shortly after illumination the frequency of the initial maximum is considerably reduced over what is obtained in response to the same stimulus when the eye is completely dark adapted.
2. The slight pause in the discharge, present in the response of the completely dark adapted eye to high intensities, is absent early in the course of dark adaptation.

Using the frequency of the initial maximum as a criterion, the course of dark adaptation of the photoreceptor was studied quantitatively in a manner analogous to that used previously by the present author in a study of this process by means of the retinal potentials from the whole eye. The results are completely comparable. Quantitatively, the curve relating initial maximal frequency to time in the dark may be fitted by the isotherm of a second order chemical reaction, following Hecht's analysis of the dark adaptation process under the same qualifications introduced in the study of the retinal potentials.

The whole of the process of dark adaptation is thus shown by the single photoreceptor unit, and its discharge of impulses quantitatively parallels the retinal action potential from the whole eye.

Cortin as a general tissue hormone. FRANK A. HARTMAN, KATHARINE A. BROWNELL and JULIA E. LOCKWOOD.

It is well known that an animal deprived of its adrenals soon shows a lower resistance to fatigue than normal. We have been able to demonstrate in adrenalectomized rats that the reflex arc, myoneural junction, and muscle are all involved. By treatment with cortical extract, a sixfold increase in resistance to fatigue has been produced in these structures. We also have evidence in man that the higher centers are affected by cortin. Patients under our observation suffering from a lowered resistance to mental fatigue have been relieved of this disturbing symptom by the administration of cortical extract. Improvement in sleep and an increased sense of well-being also indicate an effect on the higher centers. Our evidence shows that cortin plays a part in the prevention of fatigue of the nervous system that is fully as important as that played in muscle. The easy fatigue of the reflexes after adrenalectomy would account, at least in part, for the failure to produce the extra heat required when animals are exposed to cold. Adrenalectomized rats exhibit a lowered resistance to heat.

They become prostrate at a temperature in which normal animals show only discomfort. Determination of the water content of the tissues of adrenalectomized animals suggests a lower ability to shift water and thus to facilitate heat loss. These effects on the nervous system and skeletal muscle together with the effects on the digestive system and kidney indicate that Cortin is necessary for activity of the various tissues of the body. A lowered resistance to toxins, decreased metabolism and retardation of growth which occur after adrenalectomy and are corrected by the injection of cortical extract give added support to the view that cortin is a general tissue hormone.

The microscope-centrifuge. E. NEWTON HARVEY.

The microscope-centrifuge, developed in collaboration with Mr. Alfred L. Loomis, is an instrument by which a perfect highly magnified image of living cells or other material can be obtained while rotating at high centrifugal speeds. It consists of an objective lens system, which rotates with the material, and reflecting prisms to bring the image to the axis where it is observed by a stationary ocular. Illumination may be a synchronous high potential discharge in mercury vapor or the image of a tungsten filament. By slight modification the device can be used up to any centrifugal forces that transparent materials will stand. The rate of movement of individual granules in cells, from which viscosity can be determined, and deformation in shape of cells, from which surface forces may be calculated, are easily observed.

Studies on the possibility of gluconeogenesis from fat. III. The respiratory metabolism of the pig on a high fat diet. ESTELLE E. HAWLEY and JOHN R. MURLIN.

Since the pig so readily forms fat from the carbohydrate of its food, it was selected as the experimental animal in a problem designed to procure, if possible, evidence that the reverse reaction can take place.

Previous experiments on human subjects living on very high fat diets indicated that when large amounts of fat, containing a liberal percentage of lower fatty acids, were ingested, a small amount of gluconeogenesis occurred. The R.Q. was depressed below the theoretical quotient for the burning of fat. Varying amounts of acetone bodies were produced and excreted but even when the R.Q.s were corrected for this incomplete oxidation, there was evidence for formation of oxygen richer substances.

These experiments were repeated on a pig. Its diet consisted of increasing amounts of fat. The fatty acid to glucose ratios were carried as high as 6:1. Even at this high ratio there was no acidosis present as evidenced by the urine, though there was a trace present in the blood.

Lueg and Flaschentrager, in experiments determining N minimum, report that when high fat diets with an FA:G of 7:1 were fed, there was no acidosis in the pig.

On these high fat diets this pig showed the same alteration in metabolism as the human subjects on their high fat diets; namely, a low quotient following the meal with a subsequent rise above the original level.

The excretion of inorganic sulphates in man. J. M. HAYMAN, JR.

Inorganic sulphates were estimated in the blood by Power and Wakefield's method, and the urine by Fiske's benzidine method. Inorganic

sulphates are less concentrated by the human kidney than creatinine, and usually less concentrated than urea. In accordance with the belief in filtration and reabsorption, this is regarded as evidence that some sulphate diffuses back through the tubule cells. After intravenous injection of sodium sulphate, the concentration ratio approaches that of creatinine. In nephritis with elevated serum sulphate, the excretion resembles that in a normal individual after injection of sulphate.

The dark adaptation of different retinal areas. SELIG HECHT, GEORGE WALD and CHARLES HAIG.

The decrease in threshold which the eye shows during dark adaptation proceeds in two sections sharply separable in time and characteristics. The first ends in about 3 minutes, during which the threshold drops to about $1/50$ of its initial value. The second part then begins, and terminates in about 30 minutes during which the threshold drops to about $1/50,000$ of its initial value. The first part is probably determined by the cones; the second, by the rods.

With central fixation, and white light, the relative magnitude of the two parts of dark adaptation varies with the size of the measuring field. Using small, foveal fields, only the first part is evident. As the field is increased beyond the rod-free area, the first part decreases somewhat in threshold level and reaches it more slowly. The second part, however, undergoes extensive changes; it starts sooner, and goes lower, as the field increases. The time course of the second part is the same for all areas; it is merely the thresholds which are uniformly decreased.

This decrease in final threshold of the second part is due to the inclusion of more sensitive peripheral regions with increasing areas. A very small field yields a final threshold which is the same as that secured with a centrally fixated field whose radius corresponds to the peripheral displacement of the small field. Similarly, the changes in speed of the first part of adaptation are due to the inclusion of parafoveal and peripheral cones, and are duplicated in the adaptation of small fields placed peripherally when the measurements are made with extreme red light to which the cones alone are sensitive.

Lactic acid accumulation in sugar non-irritability. A. H. HEGNAUER.

The high O_2 consumption exhibited by muscles in isotonic sugar solutions is paralleled by a progressive increase in lactic acid concentration up to four or five hours at room temperature. Comparison between the increased concentration of lactic acid in sugar solutions under atmospheres of O_2 and N_2 reveals that the amount removed during any given period in O_2 , even if completely burned, accounts for only one-third of the O_2 consumed in the same period. It had been demonstrated (Fenn) that the high O_2 consumption exhibited by such muscles is reduced to normal resting levels or below by addition of a trace of KCl or NaCl to the sugar solution bathing the muscle. It is found that the addition of these salts has no effect on the rate of glycolysis in N_2 . The rate of lactic acid accumulation in O_2 is unaffected by addition of KCl, although the O_2 consumption is reduced. In the presence of NaCl, on the other hand, lactic acid continues to accumulate in O_2 , but at a slower rate. The oxidative quotient after addition of these salts becomes 5 or greater. The rate of O_2 consumption is therefore not determined by the lactic acid content.

Glycolysis is greatly reduced at 2°C., and in the presence of O₂ the lactic acid increase in muscles bathed in sugar solutions is brought back to the resting level in about four hours. Although irritability is lost in muscles immersed for an hour or two in sugar at room temperature, it is retained for at least fifteen to twenty hours at 2°C.

Further studies on the heart and median cardiac nerve of Limulus. PETER HEINBECKER.

Experimental evidence will be presented to indicate that the heart of *Limulus polyphemus* consists of an atrium and a ventricle, each of which is contractile. The form of the electromyogram shows that the final contracted state is reached by a process of successive additions. The electro-neurogram of the median cardiac nerve shows two groups of oscillatory discharges. One begins 30 to 80 sigmas before the atrial and the other 30 to 80 sigmas before the ventricular contraction, electrically recorded.

In the adult heart, activity is directly neurogenic in origin. The heart muscle can also contract rhythmically without the intervention of cardiac ganglion cells. Under such circumstances conduction is peristaltic in type, in striking contrast to the condition in the normally innervated heart where contraction is practically simultaneous throughout its length. The chronaxie of the denervated ventricle (in air) when stimulated by point electrodes is of similar order to that of the chronaxie of the normally innervated ventricle.

The effect of the extrinsic nerves on the activity of the cardiac ganglion cells has been studied. The effect of the "vagus" fibers is to lower the amplitude of the potential, to slow its oscillatory rate and to shorten its duration even to the point of extinction. The effect of the "sympathetic" fibers is to increase the amplitude of the potential, to increase the frequency of the oscillatory discharges and to lengthen their total duration. A possible explanation of the manner in which the extrinsic fibers act on the cardiac ganglion cells and also of the manner in which the cardiac ganglion cell types affect each other will be offered.

Studies on albuminuria following exercise. FRANCES A. HELLEBRANDT and ELIZABETH BROGDON.

The incidence of post-exercise albuminuria in women was studied and it was found to occur in 57.5 per cent of 40 cases in whom albumin was not found in the urine before exercise. The benign albuminurias are usually considered to be due to a mechanical interference with circulation or to asphyxiation secondary to a diminution in the amplitude of the pulse pressure. Post-exercise albuminuria was studied in its relationship to the negative phase. After long, severe bouts of exercise which produce a protracted negative phase, the pulse pressure fall in the cases with post-exercise albuminuria was 90.1 per cent greater in depth and remained sub-normal 41.8 per cent longer than in the cases without albumin following physical exertion. After exercise of speed, albuminuria was uniformly unrelated to the fall in pulse pressure. Quantitative estimations of the rate of working were made and a new electrodynamic brake bicycle ergometer was developed. Albuminuria was then studied in its relationship to the speed of doing work. Equal five minute bouts of work were done at high and low speeds. The average amount of albumin after work at high speed was 70 mgm. /100 cc. of urine as compared with 14.8 mgm./100 cc. follow-

ing work at low speeds. Both the albuminuria associated with a protracted negative phase and that following rapid work are thought to be due to increased acidity, one to prolonged localized asphyxiation and the other to the systemic accumulation of lactic acid which follows upon exercise of speed.

Chronaxie and blood sugar. ALLAN HEMINGWAY and ESTHER M. GREIS-HEIMER.

Earlier investigations by one of us (G) working on reflexes in dogs, gave indications of a relationship between excitability and the blood sugar level. The present experiments were performed on normal students. The chronaxies of the first dorsal interosseous muscle and the superficial radial nerve have been measured at various time intervals before and after the oral administration of glucose to a fasting subject. Chronaxie apparatus designed by Baldes has been used. Preliminary experiments indicate a lengthening of the muscle chronaxie at the peak of the glucose tolerance curve with a subsequent shortening. There is little or no change in the chronaxie of the sensory nerve during the glucose tolerance test.

Two stages in the effects of decreasing oxygen. YANDELL HENDERSON and ELLEN M. RADLOFF.

Investigations by physiologists have generally shown that under decreased oxygen there is over breathing, and a consequent rise of the pH of the blood; that is, alkalosis. This is particularly the case in mountain sickness.

Investigations by biochemists, on the contrary, have generally shown an increase of the lactic acid in the blood and a lowering of the pH; that is acidosis. The observations have generally been on animals in acute experiments.

We have observed the effects of progressive decrease of oxygen until death in asphyxia in dogs rebreathing air through alkali. Both of the above conditions always occur. First there is alkalosis, then acidosis. The first of these stages occurs while the partial pressure of oxygen in the air breathed is above 8 per cent. The second is the stage that occurs after the oxygen falls below 8 per cent.

These facts indicate that lactic acid and lowering of pH are not the cause of the hyperventilation in the first stage as required by the theory of Winterstein and Gesell. Even in the second stage the lactic acid and low pH are of much less importance than the decrease of carbon dioxide in the blood and tissues; for inhalation of carbon dioxide diluted in air (which must temporarily intensify the acidosis) is known to effect rapid resuscitation.

The differential physiological effectiveness of 165 KV and 700 KV x-rays and gamma rays. P. S. HENSHAW.

Interest has arisen in regard to the comparative effectiveness of different qualities (wave length) of radiations in producing certain physiological changes in living material. For a partial investigation of this problem, we have had at our disposal 165 KV x-rays produced by an ordinary deep therapy tube, 700 KV x-rays produced by the new Coolidge 900,000 volt x-ray tube (installed by the General Electric Company), and gamma rays of radium (estimated to be the equivalent of x-rays produced at one and

a half million volts) from a 4-gram element pack. The quality of x-rays, directly related to voltage, may be expressed in volts for the purposes of comparison. Wheat seedlings and *Drosophila* eggs were selected as test objects because large numbers of individuals could be irradiated conveniently at the same time and because each type of material gave a response to the radiations which could be measured with a fair degree of precision. Wheat seedlings were irradiated after a short period of germination and measured later for the linear growth of certain embryonic parts, the irradiated being compared with the controls. *Drosophila* eggs were irradiated soon after being laid and the number which survived to the hatching stage was determined by actual count. Two reactions were thus followed in order that effects produced in one could be compared with and expressed in terms of the other. The intensities of the three types of radiation were therefore adjusted to give the same reduction of linear growth in the same duration of exposure. Equal exposures of these same intensities were then given to *Drosophila* eggs to find if each kind of radiation produced equal killing effects. It was found that they did not and that they were more effective in the following order: 165 KV x-rays, 700 KV x-rays and gamma rays (1500 KV x-rays), the gamma rays being about 30 per cent more effective than the 165 KV x-rays. This clearly demonstrates that different qualities of x-rays have selective effects on certain physiological processes.

Further studies on intestinal obstruction. RAYMOND C. HERRIN and WALTER J. MEEK.

We have previously reported that in dogs distention of a balloon in a Thiry or Thiry-Vella fistula of the ileum induces all the usual symptoms of acute intestinal obstruction. In common with other recent workers, we attributed the results to disturbances in the salt and water content of the body. A large series of blood volume determinations now substantiate this view. In addition, if a strong salt solution is added through an ileal fistula, the animal may stand distention of a Thiry loop with impunity. Later the animal succumbs if the salt is withdrawn. The mere decrease in plasma volume is not fatal if the salt content remains normal.

In our experiments there is no accumulation of intestinal contents and no sign of autolysis or gangrene in the loops. There seems to be then no evidence in support of the belief that an intoxication, bacterial or proteolytic, is the cause of the acute condition. From the first it has seemed that a nervous factor must be involved and this we have now demonstrated. Animals with denervated loops withstand the pressure of distention indefinitely. Their resistance depends on their undiminished intake of water and salt.

The rôle of distention both in actual high acute obstruction and in our experimental conditions, we believe to be as follows: The distention stimulates a marked secretion of intestinal juice, rich in salt, which either accumulates in the gut and may be vomited out, or in our fistulae, drains to the exterior. In either case the result is to dehydrate and dechlorinate the animal. This loss is not replaced for the animal soon ceases to eat and the fluid intake is correspondingly reduced. The anorexia is due to afferent sensations from the distended region as is shown by the fact that animals with denervated loops continue to take food and retain their body weight. Distention is then a prime agent in the fatal outcome for it

directly stimulates the loss of body fluids and salt and indirectly diminishes the intake.

The relation of oxidation-reduction potentials to the regulation of respiration.

ALRICK B. HERTZMAN.

The platinum electrode in body fluids (blood, aqueous humor, cerebrospinal fluid, lymph) behaves as an oxygen electrode secondarily but importantly affected by oxidation-reduction systems of unknown character and present in extremely minute concentration. Shifts in potential produced by various procedures such as asphyxia, rebreathing, administration of oxygen, nitrogen, carbon dioxide, acid, alkali, cyanide, sulphide, may be qualitatively predicted from the theory of the oxygen electrode, although in several instances the electrode behaved as though changes in oxidation-reduction equilibria other than the $O_2: OH'$ system were dominant in determining the potential. The potential changes are small and do not ordinarily indicate the liberation of electromotively active reductants into the body fluids, except in the case of drastic procedures leading to marked continued anaerobiosis in which case a large rapid drop in E_h occurs, a drop that is apparently not entirely accounted for by the direct effects of the fall in oxygen tension but is suggestive of the diffusion of electromotively active reductants out of dying cells.

Excepting the special case of changes in acidity, the changes in extracellular E_h probably reflect the direction though not the magnitude of the changes inside the cell. Increased activity of the respiratory center is accompanied by a moderate fall in E_h . If the fall in E_h becomes excessive, the center is depressed. The potential drop may precede or follow the depression of the center. In the case of a depressed center with low E_h , the oxidative recovery of the center's activity is accompanied by increased E_h towards the usual level. The correlation between respiratory rhythm and E_h does not appear to involve a causal relationship but rather seems to depend on the extent to which the metabolic activity of the center is determined by the oxidation-reduction equilibria of the moment.

The physiology of respiration of fishes in relation to the environment. VI.

The oxygen and carbon dioxide dissociation curves of whole blood. TRESSA

A. HICKMAN and EDWIN B. POWERS.

Oxygen dissociation curves were determined at given carbon dioxide tensions; also carbon dioxide dissociation curves at given oxygen tensions. The blood was drawn directly from the heart or conus and kept at low temperature. O_2 dissociation curves at 0.03 per cent carbon dioxide tension differ widely from those at 2 per cent CO_2 tension. CO_2 dissociation curves rise rapidly up to 0.5 per cent carbon dioxide tension, then less rapidly up to 9.5 per cent, being irregular at the higher tensions. With these exceptions dissociation curves of blood of fishes follow the same general form as curves for mammalian blood.

Factors governing the excretion of nitrogen and fat by the bowel. HAROLD L. HIGGINS.

Under ordinary conditions, most of the fecal nitrogen comes from bacteria; any factor contributing to increased intestinal bacterial activity leads to increased fecal nitrogen. The total bulk and the water content of the stool are also factors affecting the amount of fecal nitrogen.

The quantity of fecal fat depends largely upon the normal function of the intestine to absorb fat. Minor factors which may increase fat excretion are high fat diet, excretion of large bulk (roughage), and increased calcium, acid, or alkali ingestion.

*Effects of alcohol on metabolism.*¹ H. E. HIMWICH, L. H. NAHUM, N. RAKIETEN, J. F. FAZIKAS and D. DuBois.

The effect of the ingestion of alcohol on the pH, CO₂ content and capacity, lactic acid and glucose content of the blood was determined on dogs and human subjects. Both dogs and men were in the post-absorptive condition. The dogs received 10 to 50 cc. of 19 per cent alcohol per kilo, and samples of arterial and venous cerebral blood were drawn. The human subjects received 10 cc. of 19 per cent alcohol per kilo and their blood was collected from the brachial artery.

Alcohol acts as a glycogenolytic agent, since the concentration of both glucose and lactic increased in the blood. Accompanying the lactacidemia the CO₂ capacity of blood diminished. The effects of alcohol on the CO₂ content were variable; usually there was a decrease. When the CO₂ content did not diminish there was an evident retention of CO₂ indicating a depression of the respiratory center. Following the ingestion of these doses of alcohol a decrease of pH was observed in every experiment. Values for pH and alcohol in a typical experiment was presented. The initial value was 7.41 with no alcohol in the blood. Fifteen minutes after feeding pH dropped to 7.29 and alcohol was 2.74 mgm. per cent. One hour later the pH was 7.22 and alcohol attained its maximum value of 4.10 mgm. per cent. After three hours the pH rose to 7.28 and alcohol was 3.35 mgm. per cent. In seven hours pH was 7.31 and alcohol was 2.71 mgm. per cent. In twenty-four hours the pH was again 7.41 and alcohol had disappeared from the blood. Studies of RQ after ingestions of alcohol must, therefore, take into consideration the rapid shifts of the acid-base equilibrium.

The brain removed alcohol from the blood in eight of nine observations on dogs made within one-half hour of feeding. It is of interest to note that the samples of blood returning from the brain both before and after alcohol ingestion were more acid than arterial blood. The average arterial-venous difference before the feeding of alcohol was pH 0.10.

The effect of ethyl alcohol on the respiratory exchanges during rest, work and recovery. F. A. HITCHCOCK and RUTH M. KRAFT.

Experiments show that the respiratory quotients during rest, work, and recovery are slightly depressed by the ingestion of small amounts of ethyl alcohol. The total oxygen consumption and muscular efficiency are not significantly affected. The respiratory quotient of the excess metabolism of work is lower after taking alcohol than in control tests. This suggests that in these experiments the alcohol furnished part of the energy used by the muscles in doing work.

Osmotic relationships in the egg. EVELYN HOWARD.

The osmotic relationship between the yolk and white of the avian egg has recently aroused considerable interest because of the fundamental

¹ The expenses of this investigation were defrayed in part by a generous grant from the American Medical Association.

bearing of the observed results on the general problem of cellular energetics. Straub first called attention to the fact that to maintain the observed freezing point difference between yolk and white, the yolk must in some unknown manner produce a pressure of nearly 2 atmospheres. A. V. Hill confirmed Straub by vapor tension measurements. Needham and collaborators, although confirming Straub and Hill's conclusion, could find no evidence of any vital activity of the vitelline membrane. Grollman showed that the true freezing point of the yolk is not easy to determine, and from dialyses of yolk concluded that the observations of Straub, Hill, *et al*, were experimental artifacts and that no osmotic difference actually existed between yolk and white.

The disagreement on this important point rendered a further study desirable. It has been found that freezing point determinations on egg yolk are peculiarly difficult not only because of its high viscosity and low water content, but also because it contains material which exerts an anomalous effect on ice formation in supercooled solutions. With the use of suitable technical precautions, (especially limited supercooling, very efficient stirring, and prolonged following of the temperature-time curve) I find the yolk and white to freeze at the same point, namely 0.425 (average difference 0.001°C., range 0.000 to 0.014). Dialyses of egg yolk confirm this value. In freezing dialysates, a marked change of rate in the temperature-time curve is followed by a very slow approach to the final plateau level. Unless special precautions are observed, this change of rate might be erroneously taken as the freezing point, and would cause the calculated osmotic pressure of the yolk to appear erroneously high.

Hence there remains no necessity for postulating either a vital activity of the yolk as claimed by Straub, Hill and Meyerhof, or even a residual osmotic difference as claimed by Needham and his co-workers.

Chemical relationships in the fluids of the seminal tract. C. B. HUGGINS.

Chemical analysis of semen from normal human males showed that it is a concentrated fluid with values for the calcium, phosphorus, sugar, and non-protein nitrogen approximating 3 to 10 times those of the blood serum. Bilateral ligation of the vas deferens does not lower the values appreciably. Similar values are obtained from the fluid expressed from the seminal vesicle. Much lower values for these constituents, resembling those of the blood serum, were found in the prostatic fluid in man and in the fluid accumulating in the epididymis after vas ligation in dogs and rabbits.

The fluid in spermatoceles is very dilute and contains no inorganic phosphate ions.

The evidence indicates that the seminal vesicle in man is a concentrating organ.

The carbon dioxide content of gastric mucosa. LAURENCE IRVING and M. J. WILSON.

The CO₂ content of gastric mucosa of the dog is between 20 and 30 volumes per cent. It may somewhat exceed the CO₂ content of the smooth muscle associated with the mucosa. There is rapid postmortal loss of CO₂, which is caused in part by diffusion from the thin membranous tissue during dissection. The loss of CO₂ is accelerated by postmortal acid formation. The postmortal acid formation was demonstrated by the decreasing CO₂ combining power of the mucosa when suspended in CO₂ and air mix-

tures. Postmortal acid formation was confirmed by determination of the postmortal increase in acidity.

The rapid postmortal changes make the precise estimation of the normal condition difficult, but there is no considerable difference between the mucosa from fundic and pyloric areas, nor does previous secretory activity show a marked effect. Evidently the secretion of strong acid does not appreciably affect the reaction of the mucosa and the strong acid secreted cannot penetrate the tissue sufficiently to modify the acid base equilibrium from the condition of ordinary tissues.

Some experiments bearing on the etiology of pernicious anemia. A. C. IVY, O. RICHTER and M. S. KIM.

Two types of experiments have been performed which bear on the etiology of pernicious anemia.

A. An extract was made of the liver of a pernicious anemia patient who died from complications after receiving 42 ounces of a potent liver extract by mouth. This human pernicious-anemia liver extract when injected parenterally into three pernicious anemia patients produced a remission. The liver of a second moribund pernicious anemia patient, who died after receiving only 7 cc. of a liver extract of unknown potency, was extracted. This human pernicious-anemia liver extract injected parenterally failed to cause a remission in pernicious anemia patients. (The opportunity to perform such experiments is obviously very rare.)

The results of these two experiments indicate that the substance in liver active in pernicious anemia is either absent from, or present in low concentration, in the liver of the patient suffering from pernicious anemia; also that when liver extract is administered to the pernicious-anemia patient, the patient's liver becomes saturated quite rapidly with the active principle.

B. Castle has provided evidence which indicates that the normal human stomach contains an enzyme which acts on meat to produce a substance, which is anti-anemic in pernicious anemia, and that this enzyme is absent from the stomach of patients with pernicious anemia.

The dog does not develop pernicious anemia when his stomach is removed, although a secondary anemia may occur. Hence, the dog either does not need this enzyme reported by Castle, or intestinal digestion is "adequate," or the dog is so biologically constituted that he does not manifest the picture of pernicious anemia as it occurs in man. Since it is established that the stomach of the hog (hog's ventriculin) is potent in pernicious anemia and preliminary heating of the stomach abolishes its potency, it was decided that Castle's theory might be tested by feeding "dog's ventriculin" to patients with pernicious anemia. This was done and it was found that the pernicious-anemia patient failed to respond to "dog's ventriculin." This constitutes strong comparative physiological evidence in support of Castle's theory of the etiology of pernicious anemia.

On the mode of action of "secretagogues" (liver extract) in promoting gastric secretion. A. C. IVY and M. S. KIM.

It has been found that liver extract (Lilly no. 343) is a potent secretagogue preparation. If the extract (Fraction G, Cohn, Minot and Murphy) of 100 grams of liver is perfused through the stomach of a dog with a pouch

of the entire stomach for one-half hour, gastric secretion is stimulated to the extent that from 100 to 150 mgm. of HCl are secreted in two hours. Repeated perfusions of the extract do not lead to a reduction in the secretory response which indicates that the substance does not act by being absorbed into the blood, but promotes secretion by acting locally on nerves or causes the production of a hormone.

If the above extract is freed from histamine by appropriate treatment with Lloyd's reagent, it may be injected intravenously without influencing blood pressure. When the extract is administered intravenously (volume 30 cc.) over a period of thirty minutes, the gastric glands are excited to secrete. Subcutaneous or intraperitoneal injection is without effect. This demonstrates that a secretagogue preparation free of vaso-dilators when introduced into the blood in adequate quantities may stimulate the gastric glands.

When the extract is introduced into the intestine of a dog with a pouch of the entire stomach, gastric secretion is stimulated. Whether this stimulation is due to absorption, hormone production, or reflex has not been determined.

These observations substantiate the hitherto theoretical possibility that secretagogues may stimulate the gastric glands both by being absorbed into the blood and by local action, and indicate further that the humoral agents concerned in the excitation of the gastric glands may be both hormonal and secretagogue-like in nature. The final solution awaits the chemical analysis of the blood during digestion for a specific hormone and for secretagogues.

The effect of ephedrine sulphate on the reflexes of spinal monkeys. C. F. JACOBSEN and MARGARET A. KENNARD.

Ephedrine has been shown to increase the reflexes of spinal dogs (Johnson and Luckhardt, 1928), the rigidity of decerebrate goats (Royle, 1926) and the extensor responses of dogs after high cervical transection of the cord (Hinsey, Ranson and Zeiss, 1931). In the present investigation non-toxic doses of ephedrine sulphate were injected into monkeys following acute spinal transection (1 hour to 5 days) and chronic spinal transection (6 weeks to 3 months). It was found in acute cases in which the reflexes were absent or minimal that intramuscular injection of ephedrine sulphate produced an immediate augmentation (5 to 15 minutes) of superficial and deeply reflexes and accelerated the rate at which permanent reflexes were established, as compared to the rate of return of uninjected control animals. It was our impression also that bladder function is reestablished more rapidly following the injection of ephedrine. In chronic cases, after injection of ephedrine, the reflexes were more readily elicited and of greater amplitude. This effect was maximal in one-half to one and one-half hours and generally lasted from 24 to 48 hours. Injection of adrenaline under comparable conditions gave results which were similar, although more variable and transient.

Analysis of duodenal contents in regard to gall-bladder evacuation in man. K. K. JONES.

There has been some question in regard to the source of the dark bile in duodenal drainage after the introduction of magnesium sulphate into the duodenum.

It is established that the gall bladder concentrates the hepatic bile that enters it. Human hepatic bile contains 0.05 per cent pigment and 0.07 per cent cholesterol (approximate average). Human gall-bladder bile contains 0.5 per cent pigment and 0.4 per cent cholesterol. Accordingly there should be a marked increase in these two substances in the duodenum, only if the gall bladder empties, especially since "normal" hepatic ducts do not concentrate hepatic bile.

After passage of the duodenal sound, samples of duodenal drainage were analysed for bile pigment and cholesterol and lipase at periods before and after instillation of magnesium sulphate. Bloor's colorimetric method was used for cholesterol and Hooper and Whipple's method (modified to avoid color variations due to duodenal contents) for bile pigment. The determination of lipase gave a rough index of the amount of dilution with pancreatic juice which occurred.

The results on ten human subjects show that after the magnesium sulphate there is an average increase in pigment concentration from 0.03 mgm. to 1 mgm. per cc. and in cholesterol from 0.04 mgm. to 1.7 mgm. per cc. In one subject in whom the gall bladder had been removed nine years, there was no change in either pigment or cholesterol, the pigment before and after being 0.4 and 0.47 mgm. per cc. respectively, and cholesterol 0.24 and 0.27 mgm. per cc. respectively. In the normal subjects the highest pigment concentration observed was 3.17 mgm. per cc. and the highest cholesterol was 2.65 mgm. per cc. (subject B), the highest specific gravity in this subject being 1.016. Although the increases obtained are above the range of concentration of pigment and cholesterol in hepatic bile, it must be kept in mind that the duodenal contents are being diluted with pancreatic juice, a factor which cannot be controlled in such experiments, and can be determined only roughly by enzyme analysis.

Although we recognize that this evidence is not absolute, we believe that the most likely cause of the marked increase in the concentration of pigment and cholesterol in duodenal contents, in view of the amount of dilution with pancreatic juice that occurs in most normal subjects after magnesium sulphate instillation, is gall bladder evacuation.

Effects of cholecystokin in on the isolated gall bladder. FREDERIC T. JUNG and HARRY GREENGARD.

Using the isolated gall bladder of the guinea pig in a bath of the composition recommended for the intestine by Sollmann and Rademaekers, we have regularly obtained contractions on adding solutions of cholecystokin in. Rather high concentrations are necessary. The solutions were made to contain 5 mgm. of very pure material per cubic centimeter and were then brought to various pH's from 5.0 to 8.5 by the addition of NaOH or HCl. With a bath containing 200 cc. of fluid, the addition of 3 to 10 cc. of such cholecystokin in solutions gave contractions throughout this range of pH's. This is fifty times the amount of cholecystokin in needed to cause contractions in the gall bladder of the barbitalized dog by intravenous injection. The contractions we obtained resembled those seen in the gall bladder *in situ* in the dog in their relative slowness and in the fact that at the height of the contraction a marked rhythmic activity is likely to develop. They differ from those produced by acetyl choline and pilocarpine in persisting after the administration of atropine. They have been obtained also in Tyrode's and in Locke's solutions. Strips of ileum

have behaved exactly like gall bladder throughout these experiments excepting that they do very poorly in Locke's. Other substances than cholecystokinin were found to cause contractions if made up at $\text{pH} = 8.0$, but at 7.0 and 5.0 only the cholecystokinin preparations were active.

*The effect of the heart's position on the electrocardiographic appearance of ventricular extrasystoles.*¹ L. N. KATZ and W. ACKERMAN.

Extrasystoles were induced in dogs in seven experiments from four fixed points of the heart, corresponding to the right base, right apex, left base, and left apex. The appearance of the electrocardiographic deflections of these extrasystoles were compared in different positions of the heart, viz., 1, the horizontal with the one in which the heart was shifted up to an angle of 50° with the horizontal; 2, a position in which the heart formed an angle of 30° to the left of the longitudinal axis of the body with a similar position to the right of this axis; 3, a position in which the heart formed an angle of 15° to the left with the long axis of the body and at the same time was rotated 30° on the heart's long axis, making the right ventricle more anterior, with a similar position to the right of the long axis of the body and a rotation on the heart's long axis making the left ventricle more anterior, the rotation from the previous position being 60° . In each position the extrasystoles from the four regions were induced in succession while each lead was taken.

The amplitude of the major initial deflection in each lead was measured in each of the extrasystoles and in the sinus beats and the measurement corrected for standarization. It was found that as the apex of the heart was elevated the amplitude of the normal and extrasystolic complexes decreased. In addition there was a variable shift in the direction of the "manifest potential" either to the right or to the left. Surprisingly, in a single experiment the shifts of the "manifest potential" of the various extrasystoles were not quantitatively related to one another or to that of the normal beat. The lack of quantitative relationship of the change in direction of the "manifest potential" of the various extrasystoles also was found when the apex of the heart was shifted from left to right, both with and without a rotation on the heart's long axis. Indeed, some of the extrasystoles showed a shift of the "manifest potential" in a direction opposite to the other extrasystoles.

Rotation of the heart on its own long axis, such as might occur in man in turning from the left to the right side, usually reversed the movement of the "manifest potential," causing it to move in a direction opposite to the change of the axis of the heart. This observation may explain the results that Nathanson² reported when his patients turned from the left to the right side.

The lack of parallelism in the amount and direction of the movement of the "manifest potential" found among the extrasystoles in a single experiment shows that the anatomic axis of the heart is not the only factor involved in these rotations. Another influence arises from the fact that in shifting the heart's position an alteration occurs in the effect of electrical disturbances of various regions of the heart in the plane formed by the

¹ Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

² Nathanson, M. H. Proc. Soc. Exper. Biol. and Med., 1931, xxviii, 766.

three leads. The electrical disturbances in the heart act in three dimensions; their influence on the plane of the three leads varies monotonically with the inverse of the angle they form with this plane. In changes of the position of the heart the angle of the various electrical disturbances with the plane of the three leads is altered, bringing into prominence the effects of new regions and decreasing the effects of some of the old ones. This influence may sometimes bring about a new balance in a direction opposite to the heart's axis.

It has been claimed on various grounds by Fahr,³ and Wilson and his co-workers,^{4,5} that the interpretation of bundle branch block in man is the reverse of that used in the so-called classical interpretation. The evidence has been based in part on a comparison of the anatomical axis of the heart in dog and man and on the configuration of extrasystoles obtained from a patient with pericarditis and a pericardial fistula. Our results indicate that it is unwarranted to attempt to derive the direction of extrasystoles from such comparisons of the anatomic axis without experiment. Furthermore, it is highly probable that the heart of the patient that Barker et al.⁵ used in producing the extrasystoles was not normal. It is therefore hazardous to deduce from their experiment that the direction of these extrasystoles can be applied to the normal human heart without further proof.

The excretion and utilization of sucrose when injected intravenously in man.

NORMAN M. KEITH, E. G. WAKEFIELD and M. H. POWER.

A solution containing 20 to 30 per cent sucrose was injected intravenously into three normal persons, and into three patients with impaired renal function. Disturbing subjective effects have been observed during the injection, but not in the experiments reported here. During the excretion of sucrose small quantities of protein were found in the urine of two of the normal persons. The amount of sucrose given varied from 0.6 to 1.6 grams for each kilogram of body weight, and of this 88 to 99 per cent was recovered in the urine. The excretion of sucrose was accompanied by mild diuresis.

In three experiments on normal persons very accurate estimations were made of the sucrose injected and excreted in the urine. In twenty-four hours 97 to 98 per cent was recovered. Estimation of the amount of sucrose in the blood plasma disclosed a gradual fall from an initial concentration of 600 mgm. to 10 mgm. in each 100 cc. in twelve hours, with a further decrease until, at the end of twenty-four hours, no sucrose was demonstrable. Simultaneous estimations of the concentration of sucrose in the whole blood and in the plasma showed that very little of the sugar entered the erythrocytes.

In contrast to the rapid excretion of sucrose by the normal person, it was found that among patients with renal insufficiency, both excretion in the urine and fall in the concentration in the blood were delayed. Sucrose was excreted in the urine for as long as seventy-two to ninety-six hours. In spite of this delay, the total amount recovered was 88 to 99 per cent.

Our findings indicate that the total amount of sucrose injected by vein

³ Fahr, G. Arch. Int. Med., 1920, xxv, 146.

⁴ Wilson, F. N., A. G. Macleod and P. S. Barker. Amer. Heart Journ., 1932, vii, 305.

⁵ Barker, P. S., A. G. Macleod and J. Alexander. Amer. Heart Journ., 1930, v, 720.

is excreted by the kidneys. Therefore, it seems that sucrose injected into the blood stream cannot be utilized by the general tissue cells. Hydrolysis in the body would appear to be limited to the intestinal tract.

The superior colliculi and the pupillary light reflex in the cat. ALLEN D. KELLER and LESTER STEWART.

Complete bilateral destruction of the superior colliculi, when done without injury to related structures ventrally and cephalically, does not alter the constrictor response of the pupils to light. In experiments where trauma is minimized, the reflexes are brisk and normal by the time the animal is undraped (nembutal and amytal anesthesia). By the 2nd to 3rd day, varying in different experiments, the response becomes slightly sluggish. In a few instances it is doubted that any secondary sluggishness occurred at all. In experiments where trauma was more extensive, the response to light was practically absent following operation, disappeared entirely for a few days, gradually returning to normal as postoperative time elapsed.

Some factors affecting spinal reflexes. C. E. KING, W. E. GARREY and W. R. BRYAN.

The effects of acidosis, alkalosis, and anoxia on the knee jerk and ankle flexion were studied. Barbitolised and decerebrated dogs were used. In one group the spinal cord was left intact, and in another, sectioned at the level of the last thoracic segment. Both acute and chronic preparations were studied.

The following conclusions were reached:

1. The diminution in the knee jerk and ankle flexion following the administration of carbon dioxide takes place at a much lower tension of the gas in dogs with the cord intact than in those in which it has been sectioned. The initial diminution of reflexes in the intact animal is not due to a direct depressant effect on the spinal centers, but to an inhibition associated with an excitation of centers higher up in the neuraxis.

2. The direct effect of carbon dioxide on the spinal centers is a primary excitation followed by depression.

3. The lower spinal centers are affected qualitatively like the medullary and other higher centers, but in no way commensurate quantitatively.

4. The spinal centers are more sharply affected by anoxia than by carbon dioxide.

5. An augmentation of spinal reflexes is associated with the establishment of a state of alkalosis.

6. The effects of acid-base changes on the spinal reflexes, within normal limits, are overshadowed by other factors of reinforcement and inhibition.

Heparin inhibition of "activated" and normal tissue extracts. JOSEPH T. KING.

It has been found that the addition of 1 volume rabbit plasma to 60-100 volumes of chick extract greatly increases coagulating power of the extract.¹

It has also been found that heparin exercises a much greater inhibiting influence on thrombin than on tissue fibrinogen when tested on recalcified citrate plasma.²

¹ King, J. T. Arch. f. Exp. Zellforschung, 1931, x, 467.

² King, J. T. Proc. Exper. Biol. and Med., 1931, xxix, 280.

In this study, freshly prepared chick extract is activated by adding 1 volume of citrate rabbit plasma to 20 volumes of extract. It is then rendered isocoagulant with the original extract by dilution; if used promptly, it must be diluted at least ten times and occasionally twenty times. At this latter dilution we are dealing with one-twentieth the original concentration of the extract and one-four hundredth of the original plasma.

The two extracts are now tested in the presence of heparin on recalcified citrate plasma. Suitable controls are run before and after, without heparin, to determine that the extracts are isocoagulant and remain so during the period of the test. The activated extract shows a much greater sensitivity to heparin inhibition than does a normal extract of the same strength. When the dilution needed to render the extract isocoagulant is about 10 times the coagulation time of the activated extract is usually more than twice that of the normal extract, when tested in the presence of heparin.

The sudden and marked increase in coagulative power found in an extract after activation is caused by the appearance of a body which shows about the sensitivity to heparin inhibition as previously established for serum thrombin.

Recovery of the electrically fibrillated dog heart by electric countershock. W. B. KOUWENHOVEN, D. R. HOOKER and O. R. LANGWORTHY.

An electric current of a few thousandths of an ampere is sufficient to fibrillate the ventricles of the dog heart. As this current strength is increased a point is reached above which fibrillation will not develop. A current of one ampere will inhibit all activity of the heart during the passage of the current; it will stop normal beats and do away with fibrillation if present. After the circuit is broken the heart will resume a normal rhythm.

If the one ampere countershock is applied shortly after fibrillation has been established in an animal with circulation intact, a normal cardiac rhythm can be easily and repeatedly obtained. This may also be readily done in the perfused heart.

If the ventricles of a perfused heart are left in fibrillation for several minutes the countershock alone is sufficient to recover the heart. In experiments with circulation intact it was possible to recover a normal rhythm after several minutes of fibrillation but in such cases the beats were insufficient to reestablish the circulation. Under these conditions we have succeeded in obtaining recovery by injecting Ringer's solution plus adrenalin into the carotid toward the heart under a considerable pressure. Using this procedure animals have been successfully resuscitated after three minutes of fibrillation; arterial pulsations are felt six or seven minutes after fibrillation is established and the animals make a permanent recovery.

Studies on the synergism and antagonism of drugs. I. The non-parasympathetic antagonism between atropine and the miotic alkaloids. THEODORE KOPPANYI.

It is believed that the antagonism between atropine and the parasympathetic stimulants (pilocarpine, physostigmine) is due to the fact that atropine depresses or paralyzes the same neuromuscular or neuroglandular end-apparatus which pilocarpine or physostigmine stimulate. That the antagonism is of chemical nature, or that it concerns other than parasympathetic endings, is generally denied. Certain facts, however, came to my attention of late, which may modify the above suppositions.

A. Kleitman¹ showed that pilocarpine enhances, atropine depresses caloric nystagmus. It is also possible to *elicit* vestibular nystagmus in anesthetized or unanesthetized rabbits or cats by intravenous injections of 1 to 2 mgm. of pilocarpine hydrochloride per kgm. or by 0.3 to 0.5 mgm. of physostigmine sulphate per kgm. This nystagmus may be stopped in rabbits by injection of 10 to 20 mgm., in cats by 0.5 to 5 mgm. per kgm., of atropine sulphate. After atropinization the usual doses of pilocarpine or physostigmine fail to produce nystagmus. These drug actions and the obvious antagonism cannot be due to reflexes from the periphery, for they occur in animals with high thoracic transection of the cord and both vagi and sympathetics cut.

B. Sympathetic stimulant-effects of pilocarpine and physostigmine consist in dilatation of the pupil, withdrawal of the nictitating membrane, widening of the lid aperture and slight exophthalmos upon intravenous injection of these drugs. Dale and Laidlaw² also showed that pilocarpine painted over the superior cervical ganglion likewise stimulates the ocular sympathetics. I was able to prove definitely that these ocular symptoms are of sympathetic origin for they do not occur following full ergotization.

I found that in cats the ocular sympathetic symptoms (i.e., dilatation of the pupil, widening of the palpebral fissure, withdrawal of the nictitating membrane) produced by intravenous injection of 1-3 mgm. of pilocarpine hydrochloride or 0.2 to 0.5 mgm. of physostigmine sulphate per kgm. are *at once* reversed (narrowing of the palpebral fissure, forward movement of the nictitating membrane), if small doses of atropine (0.5-2 mgm. per kgm.) are injected intravenously. Conversely, if the animal has previously received an injection of atropine, pilocarpine or physostigmine fail to produce signs of ocular sympathetic stimulation.

It could, of course, be assumed that atropine in this instance paralyzed the sympathetic end-organs. However, atropinization, though it prevents the sympathomimetic effects of pilocarpine and physostigmine, allows epinephrine to exert its usual effects. It can be shown in the same atropinized cats that whereas repeated pilocarpine and physostigmine injections are without any effects, the intravenous injection of 0.1 mgm. of epinephrine hydrochloride promptly produces all symptoms of ocular sympathetic stimulation.

Similarly, the sympathetic stimulation of the eye produced by local application of a 10 per cent solution of pilocarpine to the superior cervical ganglion is prevented when *a*, the ganglion had previously been painted with a 2 per cent solution of atropine sulphate, or when *b*, 1 mgm. of atropine sulphate per kgm. had been injected intravenously.

All these facts strongly suggest that the antagonism between atropine and the miotic alkaloids is not limited to the parasympathetic endings and that the assumption of a chemical, or rather physico-chemical antagonism must be seriously considered.

Theoretical considerations concerning the acidity of certain areas of the brain during the administration of low oxygen. HUGO KRUEGER.

During the administration of low oxygen sufficient carbon dioxide is removed from the body to suggest that the tissues in general may turn

¹ Pfüger's Arch., 1924, ccv, 201.

² Journ. Physiol., 1912, xiv, 1.

alkaline. A consideration of some of the principal factors involved indicates, however, that certain areas of the brain may become acid.

An analysis of the evidence from various authors indicates that the energy liberated by the formation of lactic acid under anaerobic conditions in muscle and brain immediately after excision is the thermal equivalent of the energy derived from oxidations in excised strips. For this reason it is assumed that the development of an oxygen debt of 1 cc. in muscle or brain is accompanied by the formation of 16 mgm. of lactic acid.

A consideration of the rate of diffusion of oxygen, the rate of consumption of oxygen, and the capillary supply of the dorsal vagal nucleus as established by Craigie¹ indicates that during the administration of 7.24 per cent oxygen² approximately 30 per cent of the tissue of this nucleus will not receive oxygen. Lactic acid will form and accumulate in the portions of the brain not receiving oxygen. A further increase in the general level of brain lactic acid should develop from the increased concentration of lactic acid found in the blood. The calculated net increase in lactic acid in the fraction of dorsal vagal nucleus not receiving oxygen will more than balance the loss in carbon dioxide due to the increased ventilation.

It must be emphasized that the above statements are theoretical first approximations based on data whose quantitative features will be improved with time.

The passage of fluid and protein through the human capillary wall during venous congestion. EUGENE M. LANDIS, L. JONES, M. ANGEVINE and W. ERB.

The loss of fluid from the blood was computed from the difference in cell volume of blood samples withdrawn simultaneously from one arm under control conditions and from the other arm at the end of a thirty minute period of venous congestion at 80, 60, 40 or 20 mm. Hg. Comparison of changes in cell volume, hemoglobin and cell counts indicated that the fluid was lost chiefly at the expense of the plasma. The change of plasma protein percentage to be expected, if no protein passed through the capillary wall, was computed in each observation from the fluid loss and the protein percentage in the control blood. By comparing the computed protein figure with that actually observed in the sample of concentrated blood, withdrawn during stasis, the loss of protein in the filtrate could be estimated.

At a venous pressure of 80 mm. Hg the fluid loss amounted to between 11.9 and 19.5 cc. per 100 cc. of blood. This filtrate apparently contained between 0.1 and 2.8 per cent protein, with an average of 1.5 per cent. At a venous pressure of 60 mm. Hg the fluid loss amounted to between 7.2 and 8.9 cc. per 100 cc. of blood. The protein content of the filtrate varied from 0.0 to 0.7 per cent with an average of 0.3 per cent.

At a venous pressure of 40 mm. Hg the loss of fluid ranged from 5.6 to 1.9 cc. per 100 cc. of blood, an amount too small to provide dependable results with reference to protein. A venous pressure of 20 mm. Hg produced no loss of fluid in one instance and in two other instances a loss of 1.1 and 2.3 cc. per 100 cc. of blood.

¹ Craigie: Journ. Comp. Neurol., 1926, xlii, 57.

² Gesell, Krueger, Nicholson, Brassfield and Pelecovich: Amer. Journ. Physiol., 1932, c, 202.

Studies were made of two patients in whom edema fluid, collecting during mild venous stasis, contained 0.09 and 0.39 per cent protein. Comparison of these figures with the corresponding plasma protein percentages indicated that in these two instances the capillary wall had retained approximately 95 per cent of the total plasma proteins.

Comparison of indirect with direct methods of measuring blood pressure in dogs. L. LAPLACE and H. C. BAZETT.

An optical oscillatory method has been used to measure pressures on one leg of a dog and allow comparison with direct records obtained from the opposite femoral artery with a Wiggers manometer. Single and double bag systems have been tested. In the direct measurement a T-shaped cannula allowing measurement of lateral pressure has sometimes been employed. By the ordinary indirect methods systolic end pressure can usually be correctly estimated, since small errors in opposite directions cancel one another; though under certain conditions serious positive or negative errors may be present. Estimates of diastolic pressure are commonly considerably below lateral diastolic pressure. With slow deflation the diastolic criterion of diminution in size of pulsation is often indefinite; with rapid deflation the criterion is more definite but the pressure within the vessel is lowered and an error is introduced. The "formoszillatorisch" criteria of von Recklinghausen¹ were difficult to demonstrate in the leg of the dog but when demonstrable that for diastolic pressures proved to be a reliable indication of end pressure. Lateral systolic pressure was usually 18 mm., lateral diastolic pressure 4 mm. below the end pressures. Attempts to correlate with cardiac output pulse pressure measurements determined from end pressures or from differences between an end systolic and lateral diastolic pressure have no theoretical justification.

The influence of flaming carbon arc radiation on blood pressure. HENRY LAURENS.

Proceeding from the demonstration that the arterial pressure of dogs may be lowered, and maintained, 15 to 20 per cent below normal, such radiation has been used on fifteen cases of hyperpiesis. In ten of these, with initial pressures ranging between 176/100 and 240/120, the pressure has been brought down to, and maintained at, considerably lower levels.

Sustained tension in skeletal muscle in relation to blood flow. C. E. LEESE and H. DAVIS.

The circulated soleus muscle of a decerebrate cat is made to record nearly isometric contractions upon a smoked drum in response to supramaximal stimuli applied to the peripheral end of the cut sciatic nerve at frequencies between 18 and 60 per second. Changes in blood flow through the muscle are inferred from the carotid pressure, which is recorded simultaneously.

Davis and Davis (in press) have shown that a steady "fatigue level" of tension is maintained under these circumstances after the first 3 minutes. The amount of tension is a function of the rate of stimulation. We have

¹ von Recklinghausen. Zeitschr. f. klin. Med., 1930, cxiii, 1.

confirmed their observation that the optimal rate for the soleus muscle is about 20 per second, higher or lower frequencies yielding lower fatigue levels.

At any given frequency of stimulation between 18 and 60 per second the fatigue level of tension is a function of the carotid pressure. The tension is very low if the blood pressure is below 100 mm. of mercury, and under these circumstances is very little influenced by changes in the frequency of stimulation. Increasing the blood pressure from 100 to 160 mm. by intravenous injection of salt solution causes an increase in the level of tension by as much as 100 per cent.

When small doses of adrenalin are administered, a pronounced increase in tension usually results. Larger doses, which cause the pressure to rise above 180 mm., cause a fall in tension. Assuming that the maintained tension is directly related to blood flow through the muscle, the inferences as to capillary changes in response to adrenalin agree with the direct microscopic observations of Hartman, Evans and Walker,¹ the failure of contraction with high blood pressure being due to local vasoconstriction.

Reflex cardiac inhibition of branchio-vascular origin in Squalus acanthias and Necturus maculosus. BRENTON R. LUTZ and LELAND C. WYMAN.

The ventral aorta of *Squalus acanthias*, with the cord destroyed, was ligated between the first and second branches and a cannula connecting with a burette, containing a physiological solution, was inserted anterior to the ligature. Cardiac inhibition was obtained when the pressure within the gill vessels was suddenly increased. An average increase of 10.7 mm. Hg above the average systolic pressure in the dorsal aorta was found to be an effective stimulus. The response is reflex, with afferent pathways in the branchial nerves and efferent fibers in the vagus. The average ventral aortic systolic pressure was 28.2 mm. Hg, the average dorsal aortic systolic pressure 15.4 mm. Hg, and the average ventral aortic pulse pressure 13.3 mm. Hg. Reflex cardiac inhibition following the increased ventral aortic pressure which results from a "spontaneous" ejection reflex lowered the ventral aortic diastolic pressure, indicating the physiological significance of the reflex.

In *Necturus maculosus* reflex cardiac inhibition resulting from mechanical or electrical stimulation could be obtained only from the gills although in elasmobranchs this response has been obtained from widespread sensory areas including the gills. A sudden increase of pressure within the gill vessels also evoked the response at a burette pressure as low as 34 mm. Hg.

Inasmuch as the threshold for the reflex in *Squalus acanthias* was found to average 10.7 mm. Hg above the dorsal aortic systolic pressure, and the average ventral aortic pulse pressure was 13.3 mm. Hg, it follows that inhibition may occur with each heart beat.

Since the carotid arteries of the mammal are derivatives of the primitive branchial system, this reflex cardiac inhibition of branchio-vascular origin may exemplify the evolutionary forerunner of the carotid sinus reflex in mammals, and a special function of the afferent branchial nerves in the reflex origin of the vagal tone of elasmobranchs and possibly of higher vertebrates is indicated.

¹ Hartman, F. A., J. I. Evans and H. G. Walker. Amer. Journ. Physiol., 1928, lxxxv, 91.

Hot-wire principle applied to measurement of blood velocity in vessels in situ.

T. E. MACHELLA.

The hot-wire principle developed by Hill¹ can be used to measure the velocities in fluid, if a heating current of 0.5 to 1 ampere is employed. A thin platinum wire (80 μ diam.) under these conditions shows fluctuations in temperature according to the velocity of fluid flowing past it. If the wire forms an arm of a Kelvin Thompson bridge, the temperature fluctuations can readily be measured or recorded on a galvanometer.

If such a wire be threaded into an artery of a dog, the system indicates the velocity changes in the cardiac and respiratory cycles. Consequently with a rapid galvanometer system it should be possible to determine not only mean velocity but the maximum and minimum velocities during these cycles without interfering with the blood stream and without the necessity of employing drugs such as heparin.

Action of some derivatives of hydroxy-benzyl alcohol. DAVID I. MACHT, FITZGERALD DUNNING and A. E. STICKELS.

Hydroxy-benzyl alcohol, or saligenin, like benzyl alcohol itself, is known to possess local anesthetic properties; and, like phenmethyolol, though in lesser degree, it exerts some relaxant action on smooth muscle. A series of chemical derivatives of saligenin were synthesized and studied particularly in regard to their relative local anesthetic and antispasmodic properties. The following compounds were prepared: amido-saligenin, nitro-saligenin, nitroso-saligenin, mono-chlor-saligenin, di-chloro-saligenin, mono-brom-saligenin, di-brom-saligenin, mono-iodo-saligenin, di-iodo-saligenin, chlor-brom-saligenin, brom-iodo-saligenin, and others. Amido-saligenin and nitroso-saligenin possess no anesthetic or antispasmodic properties. The other compounds all produced more or less local anesthesia and also relaxation of isolated surviving smooth muscle preparations. The halogenated saligenins were of special interest in that they are characterized by both antispasmodic and local anesthetic properties in a high degree, even in low concentrations. The doubly halogenated compounds were particularly active, indicating a synergistic effect.

Quantitative comparison of some muscle and nerve reactions after decerebration and decapitation, respectively. DAVID I. MACHT.

Experiments were made on white rats and on frogs. A comparison was made of the response of the vas deferens to epinephrine in two sets of rats. In one series, the animals were killed by decapitation or severing the head with a sharp instrument after anesthetization with ether; in the other, after the rats were etherized, extensive destruction of the brain tissue was effected by piercing the skull with a probe. When tested for their response to epinephrine, it was discovered in 90 per cent of cases that the vasa deferentia taken from the decerebrated rats gave a much greater contraction with a small dose of the drug than those obtained from the decapitated animals, even though the two sets of organs were of uniform length and the two series of experiments were performed at the same time.

The response of the sciatic nerves, and muscles of the thigh, in the two sets of rats to stimulation with the faradic current was also tested. In

¹ A. V. Hill. *Lancet*, 1920, ii, 752.

nearly every case it was found that the sciatic nerve retained its vitality for a considerably longer time in the decerebrated rats than it did in the decapitated animals. Stimulation of the skeletal muscles of the thigh was followed by contraction for a longer time after decerebration than after decapitation. Similar experiments on decapitated and decerebrated frogs revealed the same difference in response of sciatic nerves and gastrocnemius muscles of the thigh. When tested with a weak acid solution, the sensory nerve endings in the legs of the frogs continued to function a longer time after decerebration than after decapitation.

Comparative toxicity of aliphatic alcohols containing 1 to 18 carbon atoms.

DAVID I. MACHT and MARY E. DAVIS.

Comparative studies by both phytopharmacological and zoopharmacological methods were made on the toxicity of normal or primary alcohols with 1 to 18 carbon atoms. A number of secondary and tertiary alcohols were also included in this comparative study. It was found that the old Richardson's law, stating that the toxicity increases with the molecular weight, does not hold good for the higher members of the series, whether tested on living plants or living animals. Thus, for instance, hexyl and heptyl alcohols were less toxic than amyl while nonyl and decyl alcohols were found to be extremely toxic. On the other hand, alcohols with 11 to 15 carbon atoms were found to be much less toxic than nonyl and decyl. Alcohols with 16 to 18 atoms are solids at room temperature and could not be studied in solution even when dissolved in ethyl alcohol. Special methods of experimentation indicate that they possess mildly laxative properties for certain animals. Primary alcohols as a rule are more toxic than secondary and secondary alcohols more toxic than tertiary.

Concerning the effect of psyllium seeds on intestinal movements. DAVID I. MACHT and JAMES A. BLACK.

It is well known that psyllium seeds exert a mildly laxative effect, which has been attributed to the purely mechanical action of its mucilaginous constituents. Different species of psyllium seeds vary remarkably in their swelling power when mixed with water. Experiments were made by extracting crushed psyllium seeds with cold and hot water, respectively, dissolving out the mucilaginous material and evaporating the suspension or solution to obtain dry scales. The dried residue exhibited a lesser degree of swelling in water than the seeds themselves. Tested for their laxative effect on animals, such residue proved to be less effective as a purgative than the seeds themselves.

Extracts of psyllium seeds were made with various lipid solvents and, more particularly, with various concentrations of alcohol and with carbon tetrachloride. These extracts were studied on intestinal movements, both in isolated muscle preparations and in intact animals. When studied on surviving intestinal segments, the carbon tetrachloride extract had no effect of either stimulating or depressant character. Hydro-alcoholic extracts, especially those made with 70 per cent alcohol, distinctly stimulated contractions of isolated intestinal loops. When administered to animals, however, none of the extracts had any noticeably laxative effect. It is possible that when psyllium seeds are taken medicinally these extracts act synergistically with the swelling mucilaginous material.

The storage of water with glycogen in the liver. EATON M. MACKAY.

The old idea that approximately three grams of water are stored with each gram of glycogen in the liver was based upon very inconclusive evidence and recent investigators have failed to find any water storage with glycogen deposition in the liver. This was the result of their failure to compare the increase in water content over that of the glycogen free liver comparing instead the actual total water content with the glycogen concentration. An extensive experiment has demonstrated conclusively a definite accumulation of water along with glycogen in the liver. Due no doubt to the many other factors involved in determining the liver water content there was considerable irregularity in the amount of water stored with the glycogen but on the average this amounted to between 2 and 3 grams per gram of glycogen depending on the water content determined upon for the glycogen free liver.

The effects of partial and complete occlusion on pressures in compressed arteries. ALICE B. MALTBY.

The statement of Gladstone¹ and O. Frank and Wezler² that constriction and occlusion of a large artery during human blood pressure estimation affect the pressure proximal to the cuff to such an extent that even exact readings do not correspond to pressures in the aorta was investigated experimentally. Pressures from side branches of a subclavian artery were recorded optically and the vessel gradually compressed and decompressed between the points of registration.

The results show that true systolic pressure in the proximal end rises very little or not at all during complete occlusion and at any event returns to normal soon after small pulse waves pass the constriction. The primary oscillation of the pressure wave is greatly intensified, however, and followed by decremental after-vibrations. Since the latter are incapable of causing a pulsation distal to the constriction and since auscultatory, oscillatory and palpatory criteria for determining systolic pressure require the transmission of at least a small pulse wave through the constriction, experimental evidence does not favor the view that systolic pressure readings by clinical criteria are too high due to mechanical compression of the artery.

The physiological factors concerned in producing the transient and vibratory increase in pressure are discussed in accordance with the water-hammer hypothesis, and the compression chamber and wave motion theory of Frank.

Thermo-electric temperature studies in abdominal viscera of normal rabbits.

FRANK MARESH.

Under aseptic surgical procedures, thermo-couples were placed permanently into the active masses of the liver, spleen, kidney, stomach, intestine and lumbar muscles of rabbits. Although the insulations employed protected the thermo-couples from corrosion for only 8-10 days, the rabbits lived indefinitely with the wires within their bodies. Temperature studies were made only after the wound had healed completely. Intravenous injections of barbiturates produce an immediate and progressive fall in the tempera-

¹ Gladstone, S. A. Bull. Johns Hopkins Hosp., 1929, xliv, 83.

² Frank, O. and K. Wezler. Zeitschr. f. Biol., 1931, xci, 439.

ture of all organs; intravenous injections of pyretics require a latent period of 10 to 20 minutes before the temperature begins to rise. Intravenous injections of maltose, fructose, and galactose produced a pyrexia consistently; the maltose temperature curve shows 2 maxima, fructose and galactose show a single maximum; the temperature rise and duration of the pyrexia is independent of the dosage after a threshold value is exceeded. Lactose, sucrose and glucose produced no elevation of the temperature. Injections of epinephrine were inconstant in their action in producing a temperature rise. During the interval of temperature rise, the kidney temperature exceeds that of the other organs; the temperatures in muscles were 0.5–1°C. lower than in the abdominal organs and showed slower and smaller changes. From the similarity of the temperature curves following the intravenous injections of many substances in various concentrations, it is apparent that the energy released during a temperature rise comes from the body and not from the metabolism of the injected substance; this substance merely releases energy present in the body.

The post-operative course of dogs dehepatized by a simple one-stage method.

J. MARKOWITZ, WALLACE M. YATER and W. H. BURROWS.

In the method described the liver is removed after a 24 hour fasting period in one stage as follows: A paraffined glass cannula is inserted into the inferior vena cava just below the liver. The cannula extends as far as the thoracic portion of the inferior vena cava. A straight Eck fistula is performed below it. The hepatic vena cava is tied just below the diaphragm, after which the liver is excised.

The post-operative course is in general identical with that of dehepatized dogs prepared by the multiple-stage method. Recovery is rapid. Three points are here being emphasized: 1. There is a great tendency of intraperitoneal oozing. In some cases the fluid is merely an ascitic transudate containing erythrocytes. 2. The advent of marked hepatic insufficiency is usually accompanied by vomiting, which may be intractable. 3. When the dog has previously fasted for about 48 hours the survival period is much longer than when the liver is removed after an over-night fast. This is presumptive evidence that the ultimate cause of death is an abnormal endogenous metabolism of protein.

Respiratory metabolism and pulmonary ventilation in the tuberculous subject.

M. ELIZABETH MARSH.

A study was made of the metabolism and of the pulmonary ventilation of eight normal subjects and nineteen afebrile, non-toxic tuberculous subjects, both in the basal condition and again one hour after breakfast. The volume of expired air was collected and measured in a spirometer and later analyzed for CO₂ and O₂ in a Haldane gas analyzer.

When compared with the Aub-DuBois standards, the average basal metabolism of the normals was the same as that of eleven subjects with moderately advanced tuberculosis and of eight with far advanced tuberculosis; i.e., minus 9 per cent, minus 8 per cent and minus 8 per cent, respectively. The average pulmonary ventilation in liters per minute was 5.69, 5.57 and 6.05 for the three groups respectively. Thus it appears that both basal metabolism and pulmonary ventilation are entirely normal in afebrile, non-toxic cases of tuberculosis and bear no relationship to *extent* of lesion.

The three groups showed an average increase in metabolism after breakfast of 13 per cent, 16 per cent and 17 per cent respectively and an increase in ventilation of 8 per cent, 18 per cent and 18 per cent. The normals increased their ventilation by less than two-thirds the amount of their percentage increase in O_2 whereas in the tuberculous subjects the percentage increase in ventilation was slightly greater than the percentage increase in O_2 . This is a small difference but with exercise, when the increases in O_2 absorption would be so much greater, it would undoubtedly play a considerable rôle.

On the emptying of the gall bladder. AMEDEO S. MARRAZZI.

By means of an abdominal endoscope the size and contour of the gall bladder may be observed directly in unanesthetized dogs.

The giving of fat meals, the administration of smooth muscle stimulants in amounts which have striking effects on the movements of the intestine, on salivary secretion, etc., and the giving of the cholecystokinine of Ivy did not result in visible contractions of the gall bladders in dogs so studied.

In cats, two to four hours after a fat rich meal, the gall bladder was removed and the degree of emptying measured. The amount of emptying was not altered by drugs which affect smooth muscle contractions.

The negative results in these experiments throw doubt on the belief that the emptying of the gall bladder is normally a result of the contraction of the muscle in its wall.

Temperature studies in normal and suprarenalectomized rats. S. J. MARTIN and F. MARESH.

Temperature measurements were made with a thermo-couple which was inserted at least 5 to 6 cm. into the colon of adult rats. The colonic temperature observed on 335 normal female rats showed a most probable temperature of 38.2°C . with 50 per cent of all determinations lying in the range $37.8^{\circ}\text{--}38.6^{\circ}\text{C}$.; the most probable temperature of 193 male rats was slightly lower. The temperature distribution was the same for variations in weight, age, season and time of day. The vaginal temperature closely followed the fluctuations of the colonic temperature and was always about 1°C . below it. No correlation could be established between the vaginal temperature and the different stages of oestrus.

The colonic temperature of completely suprarenalectomized rats remained normal until 24 to 72 hours before death; in the terminal stages, it declined rapidly. In all control animals, the temperature was normal indefinitely.

Control rats were subjected to a ten minute period of ether anesthesia. The colonic temperature, taken before, during and after the procedure, showed a recovery to normal in about 2 hours in all cases. Experimental rats were similarly treated on different days after the 2nd suprarenal was removed. It was found that 1, some rats failed to recover; 2, others showed an attempted recovery and subsequently died, and 3, another group showed a normal or delayed recovery. No rats survived etherization after the 10th to 12th day following complete suprarenalectomy.

The body weight and colonic temperature were maintained beyond the survival period in some completely suprarenalectomized rats injected with the Swingle-Piffner hormone (obtained by Doctor Hisaw from Parke, Davis & Co.). In one case, such an injected animal showed a typical

normal recovery when given the ether rausch. The animal died in a few days when daily injections were stopped.

Synthesis of carbohydrates, lipids and proteins from inorganic salts, carbon dioxid and water in the absence of chlorophyl and light. S. O. MAST and D. M. PACE.

The colorless flagellate, *Chilomonas paramecium*, appears in great numbers in cultures containing hay, wheat and the like. Under these conditions it reproduces rapidly and contains relatively large amounts of starch and fat. We cultured it in various solutions on depression slides.

In water containing $\text{NaC}_2\text{H}_3\text{O}_3$, NH_4Cl , MgSO_4 and K_2HPO_4 , we obtained 166.6 generations in 49 days, 3.4 divisions per day.

In this solution with Na_2SiO_3 added, we obtained, during the same time, 3.9 divisions per day.

In water containing NH_4Cl , MgSO_4 , K_2HPO_4 , Na_2SiO_3 and CO_2 one part to four parts of air, we obtained 92.5 generations in 25 days, 3.7 divisions per day. The individuals produced contained an abundance of starch and fat. Light is not necessary for growth in this solution.

If all the individuals produced in this experiment had been retained and cultured, there would have been, at the close of the experiment, 2.46×10^{26} . The volume of these would have been 6.12×10^{11} cu. m. and in this there would have been 1.94×10^{10} cu. m. of starch and 5.98×10^{10} cu. m. of fat.

In the above solution without increase in the carbon dioxid, the fission rate was normal the first day, but the individuals were small and they contained but little starch and fat. After this the fission rate decreased rapidly and the individuals became smaller until they disappeared about the fifth day.

In this solution with increase in CO_2 but without Na_2SiO_3 , the fission rate was normal but the animals contained very little starch and fat and soon became extremely small.

In solutions containing nitrates in place of ammonium compounds, no growth was obtained.

The silicate promotes growth. It probably acts as a catalyst. The energy used in synthesis is probably obtained from oxidation of ammonia.

Fate of the thyroid hormone in experimental hyperthyroidism. F. MATHIEU and B. O. BARNES.

Since reports had been made that thyroxine was excreted largely through the gall bladder, this study was undertaken to see if the excreted compound was still active. Six dogs were fed thyroid and the bile collected. The bile was then administered to other dogs which were trained for basal metabolism tests. No increase in basal rate was obtained. The iodine content of this bile was found to be low. The experiment is being repeated using thyroxine instead of desiccated thyroid. Urinary studies are also under way.

The control of body temperature in the rat in a high frequency electrostatic field. JAMES W. MAJOR.

The rise and fall of body temperature in rats submitted to an approximately constant high frequency electrostatic field (10,000,000 cycles) has

been followed from minute to minute. The form and peculiarities of the temperature curves so obtained are discussed.

Rhythmical arterial expansion as a factor in the control of heart rate. F. D. McCREA.

Reflex alterations in heart rate cannot arise merely from stretching the arterial wall, since pressure changes with each pulse exceed effective mean pressure changes. We must assume that either a relatively constant stimulus is produced by alteration of mean pressure, or pulse pressure variations cause periodic stimulation. The effect of these variations is shown by the following experiments:

Intravenous saline infusions: In 3 experiments mean pressure was unaltered, and pulse pressure increased 5, 5 and 10 mm. Hg; the heart was slowed 0, 0 and 8 beats per minute respectively. Out of 16 experiments showing mean pressure changes of +7 to -17, 11 with pulse pressure increases of +7 to +16 mm. showed retardation of 5 to 11 beats a minute, 3 showed no change and in 3 the heart accelerated 3 to 13 beats a minute.

Aortic insufficiency: Out of 29 experiments showing practically no alteration of systolic pressure, a fall of mean pressure of 2 to 23 mm. and increase of pulse pressure of 9 to 47 mm. the heart slowed 5 to 12 beats per minute in 18, was unchanged in 9 and accelerated 5 beats per minute in 2. Increasing mean pressure toward the control level by aortic compression always resulted in a farther marked slowing.

Sinus caroticus perfusion. In 8 experiments with mean pressure changes of ± 2 mm. and pulse pressure increases of 24 to 66 mm., the heart slowed 5 to 16 beats per minute. Out of 21 experiments with mean pressures decreased 6 to 51 mm. and pulse pressures increased 11 to 75 mm. the heart slowed 6 to 8 beats per minute in 13, and was unchanged in 4. When acceleration occurred, mean pressure fell to approximately the same extent as pulse pressure increased.

Oxygen, glucose and lactic acid absorption by mammalian heart. D. A. McGINTY and A. T. MILLER, Jr.

An isolated dog heart was perfused through the aorta by blood from the carotid artery of a second dog. Perfusion rate was measured by recording the volume flow of blood from the coronary sinus. Venous blood was returned to the donor dog at constant pressure. At 3 to 6 minute intervals, simultaneous samples of arterial and of coronary venous blood were withdrawn and analysed for oxygen, carbon dioxide, glucose and lactic acid. At least 12 pairs of blood samples were taken in each experiment.

In 7 experiments prolonged for 1 to 2 hour periods during which time conditions were essentially the same, the average oxygen consumption was 2.9 cc. per gram heart per hour. The mean volume flow of blood in the coronary vessels was 46 cc. per gram heart per hour. In 4 experiments, the average lactic acid absorption amounted to 1.0, 1.2, 1.9 and 2.3 mgm. per gram heart per hour, respectively. In the same experiments, glucose absorption was 0.2, 0.02, 0.4 and 0.1 mgm. per gram heart per hour. In 3 experiments neither lactic acid nor glucose was absorbed in appreciable amounts.

It is concluded that under these experimental conditions, glucose at normal concentrations in the blood does not pass into heart muscle. Lactic acid, on the other hand, is readily absorbed by the heart except during

impaired oxidations in the muscle when lactic acid concentration there may equal or even exceed that of arterial blood. In the 3 experiments in which lactic acid was not absorbed, it was evident in 2 that the perfusion was unsatisfactory. Whether absorbed lactic acid is oxidized or converted to glycogen cannot be determined readily from these results. The R.Q.s varied from 0.55 to 0.86.

The rôle of vitamin A in phospholipid metabolism. BETTY R. MONAGHAN.

Measurements carried out in Warburg respirometers demonstrate that vitamin A, and to a lesser extent its precursor carotin, prevent the oxidative destruction of linolic acid *in vitro*. This suggests the possibility that vitamin A may be specifically concerned with the utilization of unsaturated fatty acids in the animal organism. To test this hypothesis, measurements were made on the total amount and on the degree of unsaturation of the acetone soluble and of the acetone insoluble (phospholipid) fatty acids from tissues of vitamin A deficient rats. When the results so obtained are compared with similar measurements on tissues of normal controls and of rats suffering from various other types of inanition, it is found that the amount of phospholipid fatty acids is greatly reduced in vitamin A deficient animals, although the iodine number seems to be little affected.

Regulation of sodium by the muscle. R. MOND and H. NETTER.

The muscle fibres of frogs as well as of mammals and men contain sodium in amounts up to about 30 mgm. per cent. This has been determined in frog muscles either by calculating the difference between the sodium content of the whole muscle and the amount of Na belonging to the interstitial spaces (calculated on the basis of the Cl content) or by perfusing frog muscles a few minutes with sugar solutions and analyzing Na directly. Both methods gave the same values. The Na amounts differ individually. The evidence leads to the conclusion that Na is bound to the surface of the muscle fibre.

If frog muscles are perfused with Ringer solution not containing NaHCO_3 , Na leaves the muscle as NaHCO_3 . This is shown by determinations of Na, Cl, HCO_3 and lactate in the perfusion fluid and by analyses of Na and Cl in the muscle. The sodium compound acts as buffer between muscle and the surrounding solution.

We found in investigations on the whole animal, using one leg as a control, that sodium does not leave the muscle during exercise—the lactic acid diffuses as free acid into the blood—but the muscle takes up Na during recovery when lactate enters the muscle. This reaction might be due to the value of the “Meyerhof quotient.” If this value is greater than 3, the amount of entering lactate exceeds the amount of formed CO_2 leaving the muscle and a corresponding amount of alkali remains in the blood which can be bound to the muscle, acting again as a buffer and vice versa. It might be suggested that the exchange of Na between muscle and surrounding fluid and the regulation of Na by the muscle depends on the value of the “Meyerhof quotient.”

The effect of arsenate on blood glycolysis. SERGIUS MORGULIS and SHERMAN PINTO.

Studies of the influence of arsenate on blood glycolysis show that the

phosphatase and the glycolyzing enzymatic systems are affected differently. The inorganic phosphates of glycolyzing blood invariably increase under the influence of arsenate, this increase being directly related to the arsenate concentration. The arsenate stimulates the blood phosphatase system. The effect of arsenate on glycolysis is also more or less dependent upon its concentration, but the effect may be either in the direction of acceleration or of suppression. This depends upon the source of the blood. We found that dog's blood responds to arsenate by a diminution of its glycolytic activity whereas in rabbit's blood the glycolysis is distinctly increased. This difference in the behavior of dog and rabbit red blood cells is observed when either isotonic NaCl or Locke solution is used as the suspension medium.

The dependence of cytoplasmic structures in the egg of sea urchin, on the ionic balance of the environment. A. R. MOORE.

The eggs of the sea urchin (4 species) lose their power to form fertilization membranes as a result of being kept for one minute or longer in a solution of non-electrolyte which has approximately the same pH as sea water and is isosmotic with it. This effect of the solution of non-electrolyte can be shown to depend upon the OH ions, for it practically disappears if the solution is brought to pH 4.5. The effect is also antagonized by Ca, Mg, Sr ions in nearly equal amounts, and the required quantity of each decreases regularly as the pH number of the solution diminishes. The hyaline membrane which forms after the fertilization membrane, and closely invests the fertilized egg, has been shown to have the characters of a calcium proteinate compound. It readily breaks down in acidulated (pH 4) sea water. The effect of H⁺ ions on this layer is therefore not antagonized by the metal ions of sea water. The structure of the unfertilized egg depends upon the presence of OH ions or the alkali or alkaline earth cations. Unfertilized eggs put into a solution of non-electrolyte which has a pH of 4.5 to 6, in a few minutes show pronounced Brownian movements of the particles of the cytoplasm. The particles soon tumble out of the egg like grain out of a sack and set in a gel. On the other hand, if the solution of non-electrolyte has a pH of 8, or if it contains cations of sea water even at low pH numbers, the eggs remain apparently intact for an hour or more. If we regard the egg as consisting of concentric spherical shells of material of different chemical properties, we shall find three such spheres. Going from the outside inward there is: 1, the pre-membrane stuff which is OH⁻ labile, H⁺ stable and preserved by ions of the alkaline earth metals; 2, the hyaline layer is H⁺ labile even in the presence of sea salt ions, and is OH⁻ stable when Ca⁺⁺ is present; 3, the cytoplasmic mass constitutes the spherical core, the framework of which is H⁺ labile only in the absence of sea salt cations, and is OH⁻ stable even in the absence of alkali and alkaline earth cations.

Dehydration in hyperthermia produced by high frequency electric current. E. S. NASSET, J. W. KARR and S. B. PETERS.

In some earlier work on the effect of high frequency current on the respiratory metabolism and the blood gases of dogs, very high respiratory rates were obtained which it was believed might dehydrate the animal.

High body temperatures were produced in anesthetized dogs with current of about 10⁶ cycles per second. Sixteen animals were used, two of

which served as controls. Urine was collected from the ureters and analyzed for total nitrogen. Blood was analysed for non-protein nitrogen, urea and sugar. In some of the experiments expired water and the water content of various tissues were determined at intervals during the rise of body temperature.

The rate of urine secretion and the amount of nitrogen per cubic centimeter increases up to a rectal temperature of about 42°C. Above this temperature the rate, as well as the amount of nitrogen per cubic centimeter suffers a rapid diminution, and in many cases complete anuria ensues. The blood NPN and urea usually begin to rise rather rapidly at 42°C.; the sugar falls. The amount of water lost via the respiratory tract may be increased 65 per cent above basal. Tissue water analyses thus far completed show that blood may lose about 5 per cent and muscle about 3 per cent of water under these conditions. The liver, with the exception of one case, behaves similarly.

Further studies on the metabolism of Chinese. H. NECHELES.

1. *Surface and sitting height.* It was necessary to find out whether the H. W. formula of DuBois is applicable to Chinese, because certain body proportions of the Chinese are different from those of Occidentals. In 63 male and in 63 female Chinese the surface was determined by the H. W. formula and the linear measurements of DuBois. Good agreement was found and it is therefore not necessary to alter the constants of the H. W. formula for Chinese. Apparently there is a common correlation between H. W., and surface common to all races.

The sitting heights of 40 male and 25 female Chinese were compared to the sitting heights of German subjects of same length. The sitting heights of the Chinese were always larger than those of the Germans. Therefore Pellidisi and other standards based on sitting height cannot be used for Chinese unless the constants will be adjusted.

2. *Basal metabolism.* The basal metabolism of Chinese youths up to about 20 years is higher than that of Chinese adults, perhaps higher than that of Western youths. Above 20 years the b.m. of Chinese is as low, probably lower, than that of Westerners well adept in relaxation (collected data on 183 subjects).

3. *Specific dynamic action.* It is rather low in male Chinese below 20 years of age. In adults it is comparable to that of Occidentals. Vegetarianism or mixed diet apparently has no influence on s.d.a. in Chinese.

4. *The low b.m. of the Chinese.* In Chicago it was found in 1928-29, that the b.m. of Orientals does not drop during sleep, while that of Occidentals usually does. This was interpreted as relaxation of the Occidental during the wake state already. These experiments were carried on in Peiping with normal and Somnifen-induced sleep. The results from both groups (23 experiments on 7 subjects) confirm the results of the Chicago experiments.

The relation of the spleen to changes in the specific gravity of the blood. L. B. NICE and MARTHA LINDSAY.

The specific gravity of the blood of splenectomized rats was determined by the Barbour and Hamilton method when the animals were in the quiet state and after emotional excitement (anger and pain).

Similar observations were made on the blood of partially splenectomized rats. In this group approximately three-fourths of the spleen was removed. Care was taken to see that the blood vessels to the portion of the spleen left in the body were not injured.

The average specific gravity of the blood of the splenectomized group in the quiet state was 1050.5 while after excitement it was 1050.9. In the partially splenectomized group in the quiet state the specific gravity averaged 1049.9 while after excitement it was 1051.6.

In comparison to the above groups the blood of a series of normal rats in the quiet state had an average specific gravity of 1055 and after excitement 1061.

Our results indicate that the increase in the specific gravity of blood after excitement comes largely from the reservoir in the spleen.

Effects of flooding the medulla with Locke's solution of varying temperature upon respiration and circulation. HAYDEN C. NICHOLSON.

Dogs anesthetized with morphine and urethane were used. With the animal's head flexed as far as possible so as to widen the space between the occiput and atlas, the muscles of the back of the neck were separated in the mid-line exposing the posterior atlanto-occipital ligament. This ligament and the underlying meninges were then incised exposing the posterior portion of the medulla. A fine rubber tube was inserted through the foramen magnum for a distance of 1.0–1.5 inch, through which Locke's solution was caused to flow. A small thermometer was also inserted beneath the meninges in the path of the out-flowing fluid.

The flow of Locke's solution at body temperature over the surface of the medulla had little or no effect on respiration, arterial blood pressure or pulse rate. Lowering the temperature of the fluid sufficiently (11°C–15°C) invariably quickly stopped respiration, the cessation usually being preceded by a decrease in amplitude and increase in rate. Less marked cooling (15°–26°) usually caused a marked decrease in amplitude and increase in rate of respiration, the respiratory volume being reduced. However, in some cases both amplitude and rate were decreased while in a very few there was a slowing and deepening. Cooling the fluid almost always caused a fall in blood pressure usually accompanied by a rise in pulse rate.

Raising the temperature of the fluid 2°C–5°C above the body temperature increased the respiratory volume, this increase usually resulting from an increase both in amplitude and rate of respiration, though occasionally the amplitude remained constant or even decreased slightly. Raising the temperature almost invariably caused a fall in pulse rate, usually accompanied by a rise in blood pressure, though frequently a marked fall in pulse rate was accompanied by a fall in blood pressure.

Comparison of changes in cardiac and respiratory rhythms effected by common changes in physiological conditions. JOHN NYBOER.

This study attempts to compare changes in cardiac and respiratory rhythms effected by common changes in physiological conditions. The effects of low alveolar O₂, high alveolar CO₂, mechanical asphyxia, and of injections of NaCN, Na₂S, NaHCO₃, and Na₂CO₃ were recorded in eight types of preparations, in which the functions of the vagus nerves, stellate ganglia and adrenal glands were controlled. Morphine-urethane anesthetized dogs were used.

Carbon dioxide or sodium bicarbonate administrations usually slowed cardiac rhythm in most preparations. These substances either accelerated, slowed, or had no effect on respiratory rhythm. Sodium carbonate injections usually slowed respiratory rhythm and accelerated cardiac rhythm.

Mechanical asphyxia invariably accelerated respiratory rhythm in combined vagotomized and stellate-excised preparations with adrenals intact. If begun at the end of expiration it usually retarded cardiac rhythm; whereas if begun at the end of inspiration it accelerated cardiac rhythm. When the vagus nerves were intact, slowing of both rhythms resulted during mechanical asphyxia of either type.

Both rhythms were accelerated in vagotomized, or combined vagotomized and stellate-excised preparations, by low alveolar O_2 , NaCN, and Na_2S , whether the adrenals were intact or clamped. When the vagus nerves were intact, rhythmic responses to low alveolar O_2 and to NaCN differed in adrenal and adrenalectomized dogs; i.e., adrenal preparations showed acceleration of both rhythms, whereas adrenalectomized preparations usually showed a retardation of cardiac rhythm and simultaneous acceleration of respiratory rhythm. Vagotomy abolished these differences.

It is concluded that in denervated preparations (combined vagotomy and stellate excision) with adrenals intact or excised, the effects of low alveolar O_2 , NaCN, and Na_2S were very similar on both cardiac and respiratory rhythms. Less agreement in both rhythms was obtained with CO_2 and $NaHCO_3$ administrations. Sodium carbonate injections usually produced opposite results in cardiac and respiratory rhythms.

The cardiodynamic effects following ligation of coronary arteries. OSCAR ORIAS.

The changes in ventricular contraction following ligation of the isolated ramus descendens branch of the anterior coronary artery in the dog were studied by recording pressure pulses from the ventricular cavities, aorta and pulmonary artery by means of Wiggers' optical-manometers.

In the preponderance of experiments, ligation was followed immediately by hypodynamic beats characterized by decreased pressure amplitude and a significant reduction in the intervals of systole and in volume of systolic discharge. In consequence of the reduced systolic output, systolic and diastolic pressures fell. These effects however were promptly compensated for (4-7 min.) through an increase in diastolic size and a rise of initial tension which restored systolic pressures in the left ventricle and aorta as well as the duration of contractions to normal. Occasionally a slight overcompensation occurred so that the ventricle action as a whole was dynamically supernormal.

In approximately one-third of the experiments, systolic pressures in the aorta and ventricle were not decreased primarily, but tended to increase. In these hearts the typical initial abbreviation of contraction was always present however.

Two views as to the cause of the changes in ventricular action after coronary ligation were discussed, viz., 1, that the contractile force and duration is changed in all muscle units of the ventricle involved, and 2, that the changes observed are due to modifications or deletion of fractionate contractions in the potentially infarcted area. The conclusions were reached 1, that the primary depression of contraction and decrease in ventricular systole are caused by deletion of fractionate contractions in

potentially infarcted areas; 2, that absence of the initial depression may be accounted for by primary anoxemia of some affected muscle fractions, but 3, that the real compensation by which duration of contraction and normal pressures are restored and maintained is due to the increase in diastolic size and initial tension. Through the response of unaffected muscle the contractions of the ventricle as a whole are restored to normal.

A comparison of the catalytic activity of the ash of the different tissues. O. S. ORTH and W. E. BURGE.

Many investigators have made comparisons of the catalase content, or the catalytic activity, of the different tissues. The object of this investigation was to determine if the ash, like the tissues, possesses the power of liberating oxygen from hydrogen peroxide, and to compare the catalytic activity of the ash of the different tissues. The tissues used were striated, cardiac, and plain muscle, lung, brain, liver, pancreas, kidney, arterial and venous blood of dogs. After incinerating the tissues 200 milligrams of the powdered ash were weighed out and added to 100 cc. of neutral peroxide in a bottle and the oxygen liberated was conducted to a burette inverted in water, collected, and measured. With the use of eleven such burettes all the determinations in each series of experiments were made simultaneously. Care was also taken to see that the peroxide was neutral after the addition of the ash.

The following are the results of the average of five series of experiments. The ash of skeletal muscle liberated 477 cc. of oxygen in 72 hours, cardiac muscle 465 cc., kidney 345 cc., plain muscle 213 cc., venous blood 182 cc., liver 163 cc., lung 161 cc., arterial blood 114 cc., brain 85 cc., pancreas 37 cc., and the check, which consisted of peroxide alone, liberated 16 cc. of oxygen in 72 hours. By comparing these figures, it will be seen that the ash of skeletal muscle possessed the greatest catalytic activity, cardiac muscle next, kidney next and so on down to the ash of the pancreas, which exhibited least catalytic activity of all the tissues studied.

A study of the reflex influence of the sciatic and vagus on the motility of the bullfrog's stomach. T. L. PATTERSON.

Recent studies on the stomach of the monkey as influenced by central stimulation of the sciatic and vagus nerves have shown that the gastric response is dependent upon the pre-existing state of tonus of the gastric mechanism. If the stomach is hypertonic, reflex inhibition will follow central stimulation of these nerves; if hypotonic, a contraction may occur or there may be a definite increase in the tonus.

Stomostomized bullfrogs were employed in this investigation. They were decerebrated and either the sciatic nerve plexus or the vagus was isolated for stimulation with shield electrodes attached to the skin or urostyle. Negative results were obtained from stimulation of the sciatic plexuses, thus affording evidence that the afferent sensory fibers do not have any reflex connection via nuclei in the brain controlling the movements of the empty stomach. Complete section of the splanchnic nerves to increase the gastric tonus (This Journal, 1920, liv, 153) was also without effect. Central stimulation of the vagus after unilateral vagotomy resulted in a reflex inhibition of gastric motility but this reflex was abolished after bilateral vagotomy. No augmentation has ever been observed. In prolonged fasting (five to six weeks) a mild degree of gastric tetany was

exhibited in which groups of two to four contractions failed to return to the base line. This condition has not been observed before due to the shortness of the fasting periods.

The results of this investigation give evidence that the highly developed and complicated reflexes present in the gastric mechanism of the monkey and primarily dependent upon its pre-existing state of tonus do not exist in the simplified gastro- neuro- musculo-mechanism of the bullfrog.

The effects upon cardiac musculature of subthreshold electrical currents.

HUBERT B. PEUGNET and ARTHUR S. GILSON, JR.

Paralleling the recently published work of Erlanger and Blair in which they reported studies of the changes of nerve irritability in response to the passage of electrical currents, a similar study has been carried forward with heart muscle. In general, there is a remarkably close agreement found between the reactions of the two tissues, the major difference being that the time functions of the cardiac response curves are approximately 40 times greater than those of nerve tissue.

The irritability of heart muscle (ventricular muscle of the turtle) has been studied with respect to the effect upon it of polarizing currents of various strengths and durations, ranging from the very brief induction shock to the constant current of 100 or more sigmas in duration; and in strength, from the weakest to the just subthreshold.

Though there are minor differences in the responses to different types of polarizing currents, certain general statements may be made concerning the major characteristics of the changes, which are common to all types.

At the cathodes, the first change is always one of enhancement of irritability. The duration of this period of enhancement is variable, depending upon the duration and strength of the polarizing current. It persists in general during the passage of the current. At the termination of the current, the enhancement of irritability commences immediately to fall, but requires a definitely measurable time to reach the base line; this time is that known as the period of "latent addition" or "post-cathodal enhancement." Following immediately after the period of latent addition, there ensues a depression phase, during which the tissue is depressed below its normal state. This depressed condition has been descriptively named "post-cathodal depression." This latter phase passes off gradually, following somewhat the same curve as that of the recovery from relative refractoriness.

The time relations of the above mentioned changes vary somewhat with variation in the strength and duration of the polarizing current. Suffice it here to say that the longer the time during which a current flows, the greater is the tendency to post-cathodal depression. The induction shock gave the longest latent addition period of any of the currents investigated.

These findings are of some significance in the explanation of the behavior of the tissue in response to continued rapidly repetitive stimulation, and in the development of certain types of "block," particularly that described by Wedenski.

Improved methods for regional analysis of bone. S. E. POND.

Analytical vessels and auxiliary methods have been devised to determine the water, fat, and acid-soluble fractions of bone removed at biopsy so

as to permit the study of tissue sections from any region desired. The removal, mounting, and sectioning of bone also allows the preparations for microscopic study of thin sections, prepared serially before and after the sections used for chemical analysis. Whole bones, or pieces of calcified tissues have been successfully cut without decalcification or other chemical treatment commencing within ten minutes of removal from the body. Cutting has been done under oil, salt solution and under alcohol, by means of a small, rotary shear mounted on a lathe-miller driven by electric motor. The cutting operation is semi-automatic allowing about one minute for the full preparation of each section, varying in thickness from 10 to 500 microns, and in area from 1 cm. diameter to 5 cm. or larger. Portions selected for microscopic study of cellular content or composition were mounted on glass slides, serially, as cut, and subsequently fixed, stained or otherwise preserved as required. Portions for chemical analysis (weighing 40 to 120 mgm.) were transferred to tared Jena, filter-crucibles, fitted with ground, vacuum-tight stopper. This glass vessel for analytical purposes is supported by ground-joint and a pyrex auxiliary with three-way stop-cock to permit evacuation, solution, and transfer of solutions without loss. Suitable connections have been made for determination of CO_2 in the Van Slyke manometric analyzer. The methods are employed in studies of cancellous bone of experimental animals, but may be used for analytical and routine studies of normal or diseased bone.

The physiology of respiration of fishes in relation to the environment. X. The mechanism of the deposition of gases into the swim-bladder. EDWIN B. POWERS.

Gases are deposited by simple diffusion.

High proportions of oxygen are made possible by the relations of the two sets of capillaries in the rete mirabile-gas gland mechanism and by the fact that fish blood undergoes changes at higher carbon dioxide tensions which bring about a lowering of the capacities to carry oxygen and carbon dioxide aside from the carbon dioxide-hydrogen ion effect.

This is first brought about in the capillaries of the gas gland. The excess oxygen and carbon dioxide are given over by simple diffusion to the arterial blood in the arterial capillaries of the rete mirabile. The arterial blood is in turn modified. After repeated oxygen and carbon dioxide circuits the total gaseous partial tensions of the blood reach the mechanical pressure on the blood. Oxygen and carbon dioxide with augmented tensions pass by simple diffusion into the lumen of the swim-bladder. In case bubbles are formed they are made up of all the gases contained in the blood and in proportions determined by their respective tensions. The inert gases by means of these bubbles are deposited in the swim-bladder bit by bit independent of their respective partial pressures already in the swim-bladder. The oxygen and carbon dioxide leave the swim-bladder more rapidly than the inert gases in emptying. This increases the proportions of the inert gases in the swim-bladder above those found in the gas bubbles. Thus their partial pressures are increased, the mechanical pressure remaining constant. It follows that these inert gases would have partial pressures in the swim-bladder greater than their respective partial tensions in the blood but in the same ratios to each other. This has been known to be the case since the time of Schloesing and Richard (1896).

Homolateral synchronism of lymphatic hearts in the frog and conditions attending neurogenic-myogenic reversal. F. H. PRATT and M. A. REID.

Although the members of each pair of lymph hearts do not beat in unison, it is discovered that there is a definite synchronism of anterior and posterior organs of the same side which persists even in extreme arrhythmia but is abolished by mid-section of the cord.

In most cases standstill of an anterior lymph heart is accomplished in *R. Pipiens* by cutting a highly constant ramus (post. sup. thorac.) of the III spinal nerve, after which an independent rhythm develops which may be in turn supplanted by the neurogenic state through regeneration of the nerve.¹ Further observations have aimed to compare the characteristics of the lymph-heart muscle in these two states.

The normal or newly denervated lymph heart is passive except when the nerve is transmitting impulses. Each normal beat is known to be the tetanic expression of a group of nervous discharges.² Directly or indirectly, the muscle may be summated, tetanized and graded—the last effect showing, in its step-like partials, the type of grading influence experimentally realized in skeletal muscle.³ Complete tetanic fusion implies here a short refractory period. Curare causes typical disjunction.

A change in properties characterizes the denervated heart once established, in automaticity. Summation and tetanus are lost. Size of response is unrelated to strength of stimulus. There is a systolic refractory period; extra-contraction is followed by a post-extrasystolic pause, often nearly compensatory. The coördinated rhythm may survive both destruction of the spinal cord and curarization. The "limulus" type of control has thus been replaced by the vertebrate cardiac type. That the two are experimentally reversible is significant either of transition in fundamental characters within the muscle fibre or—a possibility under investigation—the parallel existence of two tissues of widely different potentialities.

Protein as a fuel in muscular exercise. DAVID RAPPORT and ATTILIO CANZANELLI.

Studies of the nitrogenous metabolism of protein in connection with exercise do not necessarily furnish evidence regarding the utilization of the non-nitrogenous fragments. For example, lack of increase in nitrogen excretion might indicate that deamination was not accelerated, but would not rule out the oxidation of the non-nitrogenous moiety to provide fuel for muscular exercise. We have studied the latter by means of the excess respiratory quotient. An animal was subjected either to starvation or to a high fat diet until the resulting resting and excess R. Q.s approached those of fat (0.72–0.73) on the morning of the experimental day. Then, after the ingestion of 500 grams of beef, the resting R. Q. of the animal was 0.79, indicating a practically exclusive protein metabolism. During exercise and recovery therefrom, the R. Q. remained in this neighborhood (0.78 to 0.86).

Following a maintenance diet composed equally of carbohydrate and protein, the administration of either 25 or 50 grams of glucose caused the

¹ Pratt and Reid, Proc. Amer. Physiol. Soc., This Journal, 1930, xciii, 681.

² Brücke and Umrath, Pflüger's Arch., 1930, ccxxiv, 631.

³ Pratt and Reid, Proc. XIII Internat. Physiol. Cong., This Journal, 1929, xc, 480.

excess quotient of exercise and recovery to be unity, whereas, upon the administration of 25 grams of glucose and 25 grams of meat protein, both the resting R. Q. and that of exercise and recovery were practically 0.90 (the theoretical quotient of the mixture).

It is concluded that the cleavage products of protein metabolism can be utilized to provide fuel for muscular exercise.

The anion-cation content of hepatic and gall-bladder bile. I. S. RAVDIN, C. G. JOHNSTON, CECILIA RIEGEL, J. HAROLD AUSTIN and S. WRIGHT.

Studies have been made on the anion-cation content of hepatic and gall-bladder bile. Known amounts of hepatic bile have been subjected to gall-bladder activity, so that quantitative changes could be studied.

Profound changes in the base, chloride, bicarbonate and bile salt concentrations occur when hepatic bile is subjected to gall-bladder activity. Similar experiments have been conducted on the fate of bile pigment and cholesterol in the gall bladder. We have also studied the effect of the damaged gall bladder on hepatic bile.

The changes in anion and cation concentration which occur in the normal gall bladder are not observed when hepatic bile is subjected to the influence of an infected gall bladder. Base and bile salt concentrations increase while chloride and bicarbonate concentrations decrease in normal gall-bladder bile. In the damaged gall bladder base is of about the same concentration, chloride and bicarbonate tend to increase and bile salt decreases in concentration.

Observations on the inactivation of dialyzed hemoglobin solutions. G. B. RAY and C. I. THOMAS.

Dialyzed hemoglobin solutions prepared by Adair's method were allowed to inactivate, the course of the reaction being determined by changes in the oxygen capacity. Simultaneously with these measurements spectrophotometric readings were made. The distortion of the oxyhemoglobin absorption curve when calculated as per cent of methemoglobin agreed with the loss in per cent of original oxygen capacity found by the Van Slyke apparatus. It is concluded that inactivation is due to methemoglobin formation.

The influence of irradiated ergosterol on the metabolic rate of normal dogs. C. I. REED.

Intravenous administration of irradiated ergosterol 10,000 X to normal dogs produces invariably a pronounced increase in the metabolic rate. This increase is not a function of altered nitrogen metabolism, and is not correlated with any other factor observed. It is not directly related to the dosage nor duration of administration. In some cases there was a sharp rise within twenty-four hours, in others there was a latent period of several days.

The action of corpus luteum extracts containing progesterin on uterine motility in the unanesthetized rabbit. SAMUEL R. M. REYNOLDS and WILLARD M. ALLEN.

After the first few days of pseudopregnancy in the rabbit, the uterus is both quiescent and refractory to the normal stimulating action of oestrin

(Theelin). Removal of the ovaries at this time renders the uterus readily susceptible to small amounts of Theelin.¹

In the present experiments it has been found that progestin, an active extract of the corpus luteum which induces endometrial proliferation^{2,3} is capable of inhibiting the stimulating action of Theelin (up to 1000 rat units per kilogram of body weight) provided a sufficient amount of progestin is employed (4.8–5.0 rabbit units). By the use of smaller amounts of progestin (1.6 rabbit units) the response to 100 rat units of Theelin is inhibited, but by giving 500 rat units of Theelin the normal Theelin-response may be obtained. It has also been found that progestin can overcome the spontaneous motility of the oestral (*post partum*) rabbit.

These data *in vivo* in the unanesthetized rabbit provide adequate replacement-therapy evidence that the action of the corpus luteum is inhibitory to uterine motility, and that it is antagonistic in this respect to the action of oestrin (Theelin).

The action of benzol on granulocytes. PAUL REZNIKOFF and RUTH FULLARTON.

Benzol has been considered primarily a leukocytic poison by practically all workers and most investigators believe that the granulocytes in particular are affected. If this were true we would have an excellent agent to study a cell having great importance in physiological and pathological processes.

To throw light upon this question benzol was administered in 39 experiments to 25 rabbits and 3 cats; in 34 cases, subcutaneously; in 2, by vaporization; and in 3, by mouth. The following factors were noted: 1, total white blood cell count; 2, total granulocytes; 3, immature granulocytes, and, in some cases, 4, the bone marrow.

The results are shown in the following table:

	NONE	SLIGHT	MODERATE	MARKED
Depression of W.B.C.....	7	7	20	5
Agranulocytosis.....	26	1	6	6
Appearance of immature granulocytes..	9	7	11	12

Of 12 bone marrows studied, 2 were fatty, 1 bloody, 3 showed no granulocytic depression, 5 demonstrated moderate granulocytic depression, and 1 marked depression.

These experiments indicate that benzol can usually produce a depression of the total leukocyte count, rarely an agranulocytosis, and usually calls forth immature granulocytes. This suggests that benzol produces complicated leukopoietic mechanisms and cannot be relied upon as an agent to study variations of granulocytes from the normal.

Experimental production of the grasp reflex in adult monkeys by lesions of the frontal lobes. CURT P. RICHTER and MARION HINES.

Our knowledge of the grasp reflex has been based on the observation of

¹ Reynolds, S. R. M., 1931. This Journal, xcvi, 230.

² Allen, W. M., 1930. This Journal, xcii, 174.

³ Corner, G. W., and W. M. Allen, 1929. This Journal, lxxxviii, 326.

its presence in new-born infants and in adults with tumors or lesions involving the frontal lobes or the underlying structures. These lesions have all been diffuse, involving many different structures, so it has not been possible to determine just what parts of the brain must be injured before the reflex appears.

In addition to this limitation or lack of definite knowledge regarding the destroyed areas of the brain, the observations on patients have been limited by the lack of means of measuring the reflex.

These difficulties have been overcome in the present experiments on monkeys, in which circumscribed lesions were made in different parts of the brain, and objective measurements of the reflex were obtained. The reflex was measured by determining how long the monkey will hang from a horizontal bar by the right and left hands after the operation. A normal monkey without a lesion will not hang from a bar under the same circumstances.

It was found that the reflex can be elicited consistently only by removal of the pre-motor area, area 6 of Brodmann, the medial as well as the lateral surfaces, down to the level of the corpus callosum. The removal of this area in one frontal lobe brings out the reflex in the opposite hand, but only temporarily, from one to twenty days; subsequent removal of this area in the other frontal lobe brings out the reflex on both sides, and permanently. Latent functional differences of the reflex on the two sides produced by unilateral and bilateral lesions can be brought out very strikingly with several drugs, particularly bulbo-capnine and hyoseine.

Seasonal, endocrine and temperature factors which determine percentage metabolism change per degree of temperature change. OSCAR RIDDLE, G. C. SMITH and F. G. BENEDICT.

It is shown that the basal metabolism of various species and races of doves and pigeons markedly and similarly fluctuates with the seasons. The studies are based upon animals subjected to outdoor temperatures from April to November. They are given considerable protection from cold during the winter, and are subjected to air of greatest cooling power during autumn. Other earlier studies by Riddle have shown that the thyroids of these birds are larger and apparently more active in autumn and winter, and are smallest in summer; the gonads have the reverse relation to season. Nearly 350 metabolism measurements made on a single race of common pigeons at 15°–20°–30°C. during the four seasons show that the state of the animal—attained in connection with season—markedly influences the value of the metabolism at whatever environmental temperature this metabolism is measured. Further, at the various seasons the metabolism is quite unequally affected by measurement at environmental temperature either higher or lower than 20°C.; the metabolism of birds in summer being practically the same (change of 0.4 per cent per degree) when measured at 20° or 30°, but the metabolism of summer birds measured at 15° shows an increase of nearly 3.5 per cent for each degree of temperature change below 20°. Measurements made in autumn at the different temperatures give dissimilar relative changes; between 20°–30° the decline of the metabolism is 0.8 per cent per degree, while from 20°–15° the change is 1.5 per cent per degree. Though the point has not been tested it would logically follow that the so-called "critical" temperature will vary with the seasonal (and endocrine) state of the animal.

The effect of a ketogenic diet on convulsions of experimental origin in cats.

SARAH R. RIEDMAN.

The use of the ketogenic diet as a therapeutic agent in epilepsy has stimulated interest in the problem. The object is to determine the effect of the diet on the minimal convulsive and lethal doses of absinth as compared with the dosage in control animals.

The diet which has been found least unpalatable consists of bacon and "heavy cream." The diet of control cats in the laboratory consists of fish or meat and milk, yielding about 400 calories, 35 per cent of which comes from protein, so that the animals get about 5 grams of protein per pound. The ratio of ketogenic to antiketogenic substances is approximately 1:5 to 1. Since the ketogenic diet is less palatable than this, the caloric intake is about 25 per cent lower, and the protein intake is about 20 per cent of that of control animals. The ratio of ketogenic to antiketogenic substances is approximately 3:1.

Animals are kept on the diet two weeks or longer. The presence of a good ring in the urine with the sodium nitroprusside test is taken as an indication of ketosis. The Folin method is used for total acidity.

Absinth is injected intravenously in gradually increasing doses. At present, no general conclusion can be drawn, although the minimal dose seems to be increased.

All but two of the animals have lost weight. A positive test for acetone has been obtained in all. The urinary output during the 24 hours preceding the experiment varies from 25 cc. to 60 cc. The titer for the experiment varies from 1.0 to 1.5 cc. N/10 NaOH per cubic centimeter of urine.

The effect of intravenous injection of 25 per cent acetone is being investigated in control animals. The minimal convulsive dose of absinth is first determined before and after the injection of the acetone. The minimal dose is generally raised after an interval, although the immediate effect may be a reduction. The lethal dose is generally decreased.

The mode of action of adrenin. ARTURO ROSENBLUETH.

The exponential shape of the responses of smooth muscle to adrenin may be explained on either a physical or a chemical hypothesis. It is therefore necessary to use other characteristics to determine the correct explanation.

Varying doses of adrenin produce responses which differ quantitatively. If the maximal height of the responses is plotted against the doses of adrenin, different curves should theoretically be obtained according to the process conditioning excitation: diffusion should give a straight line; adsorption, a parabola; and mass action (chemical reaction), a hyperbola. Experimental data obtained for the contraction of the nictitating membrane (isotonic and isometric), the rise of blood pressure, the increase of heart rate, and the inhibition of the non-pregnant uterus of the cat, when plotted, all produced equilateral hyperbolas.

A chemical hypothesis is therefore adopted: adrenin (A) combines in a reversible reaction with some substance (H) in the muscle; the response is proportional to the amount of the product AH present.

If the all-or-none principle were applicable to these cases, the quantitative differences of the responses to varying doses of adrenin would be due to differences of the thresholds of the individual cells. It would then have to

be admitted that the threshold might sometimes be over 600 times greater for some cells than for others, which is highly improbable. The thresholds would vary around a mean; hence the plots of the maximal heights of the responses should be S-shaped frequency curves, which is not the case. It is therefore impossible to reconcile this experimental evidence with the all-or-none law.

The metabolism of levulose: I. Some general considerations on provocative levulosuria. ALLAN WINTER ROWE, ALBERT J. PLUMMER and MARY McMANUS.

This is the first report on an extensive study on the metabolism of levulose in conditions of health and disease, paralleling similar investigations on galactose, by the senior author and associates, already reported in the literature. Strauss in 1901 suggested the use of a provocative test meal of 100 grams of levulose as an index of the level of hepatic function, using the appearance of the sugar in the urine as the criterion of positive response. The slight influence of ingested levulose on blood sugar curves renders this form of approach less significant than is the case with the widely used glucose tolerance test. Many samples of levulose of even a high degree of purity seem to exercise a marked laxative action on the intestines and this subversive factor can be excluded with difficulty. With an exceeded tolerance, levulose is identified qualitatively by the Benedict and Selivanov methods, and quantitatively measured by a combination of the Folin-Wu procedure and the polariscope. The normal presence in the urine of small amounts of left rotating material, as previously recorded by others, has been verified and correction for it applied. In cases with demonstrated hepatic dysfunction, the levulose tolerance shows + or - fluctuations similar to those recorded by the senior author with galactose as the test substance. All subjects had thorough clinical and laboratory studies in addition to the sugar testing, permitting the determination of departures from the normal which could readily be overlooked in a superficial examination. While the levulose results show much less uniformity than do those with galactose, the work already completed does not seem to indicate clearly the sex difference with levulose that has been recorded with galactose.

Simultaneous internal and external stimulation of the iris by adrenin. TEODORO SCHLOSSBERG.

The reactions of the pupil, denervated by removal of the superior cervical sympathetic ganglia, to adrenin instilled in the conjunctival sac and to emotional excitement were studied in cats.

Instillation of adrenin in the conjunctival sac begins to produce a marked dilatation of the corresponding pupil only about twelve days after the denervation. As time passes this dilatation occurs earlier, is more marked and lasts longer. Emotional excitement produces stronger and longer widening of the previously instilled pupil.

On repetition of emotional excitements the effects increase.

These reactions occur even several hours (3 to 8) after the instillation, when both pupils are apparently in identical conditions.

Inactivation of the adrenals diminishes but does not suppress the above-mentioned results.

Carotid sinus reflexes and the chemical regulation of respiration. CARL F. SCHMIDT.

Participation of sinus reflexes in the respiratory responses to physiological alterations in gas tension of blood is indicated by these observations:

1. Denervation of sinuses reduced and sometimes completely abolished the respiratory response to nitrogen inhalation in dogs, cats, and rabbits. Response to $\text{CO}_2\text{-O}_2$ inhalation was somewhat reduced in dogs and cats, unaffected in rabbits.

2. In crossed-circulation experiments on dogs and cats anoxemia in the donor caused reflex hyperpnea in recipient; $\text{CO}_2 - \text{O}_2$ had much less effect; the reflex effect of nitrogen could be abolished by inhalation of 10 per cent CO_2 by the donor. Overventilation of donor caused respiratory depression or apnea in recipient.

These "chemical reflexes" appear to arise from first part of occipital artery in the dog, while "pressure reflexes" arise from the sinuses proper. Furthermore, pressure reflexes to respiration have characteristics so different from those to circulation that separate sets of end-organs for the two sets of pressure reflexes are most probably present in the sinuses.

The effect of cyanide upon the contracture of smooth muscle. FRANCIS O. SCHMITT and PAUL A. NICOLL.

Sodium cyanide in dilute solutions may partially or completely inhibit the contracture of rabbit uteri and of intestinal strips treated with drugs such as arecolin, pilocarpine, acetyl choline etc. Using the Trendelenberg technique, the contracture resulting from the addition of a certain amount of drug was recorded, and the drug then washed out. Buffered NaCN was then added to the solution to make a concentration of M/2,000. This invariably stopped the rhythmic beating of the strip and often produced a small initial contraction. Addition at this point, of the same amount of drug as that originally used, produced no contracture. Washing the cyanide and the drug away, and testing again with the original amount of drug, a contracture resulted which was similar to that first recorded. It can be demonstrated in this way that M/2,000 NaCN may inhibit completely and reversibly the contracture of uterine strips treated with acetyl choline, pilocarpine, arecolin, adrenalin, and BaCl_2 . The cyanide may be diluted to M/200,000 and yet partially inhibit the contracture produced by 5×10^{-6} parts of acetyl choline. Furthermore, the addition of M/50,000 NaCN *simultaneously* with the drug still produced a smaller contracture than that produced by the drug alone, indicating that the seat of the cyanide action may be at the surface. Further experiments with carbon monoxide are being undertaken to determine whether the inhibitory effect is due to a disturbance of oxidative processes.

The oxygen consumption of stimulated nerve. FRANCIS O. SCHMITT.

A method is described for the simultaneous measurement of the action potentials and of the oxygen consumption of nerves. The respiration is measured by means of a differential volumeter of improved design while the action potentials are recorded by means of the cathode ray oscillograph. This technique permits of the investigation of the various electrical phenomena in nerve in relation to metabolic processes. A contribution is made

to the current controversy as to whether the extra oxygen consumption of stimulated nerve is a result of genuine metabolic phenomena in the nerve and is caused by the events which attend the physiological passage of the nerve impulse, or is an artefact due to the passage of the stimulating current, as claimed by Winterstein. Graphs are presented in which the strength of stimulating current, action potentials, and oxygen consumption of frog nerves are plotted simultaneously. These demonstrate that the curve of the oxygen consumption parallels more nearly that of action potential than that of the stimulating current.

The lactic acid cycle in the excised skeletal muscle of the diabetic dog. EPHRAIM SHORR, ROBERT O. LOEBEL and H. B. RICHARDSON.

In a previous communication¹ we reported the presence of a lactic acid-conversion mechanism in the excised skeletal muscle of the normal dog, similar to that found by Meyerhof in frog muscle. In our experiments, the ratio

$$\frac{\text{mols lactic acid disappearing}}{\text{mols lactic acid oxidized}}$$

under aerobic conditions averaged 4.3 to 1. Under anaerobic conditions the ratio

$$\frac{\text{mols lactic acid appearing in N}_2}{\text{mols lactic acid oxidized in O}_2}$$

was 3.8 to 1, i.e., essentially the same. This demonstration of the lactic acid cycle in mammalian muscle was subsequently confirmed by Boyland² in Meyerhof's laboratory working with minced ox-muscle.

We have extended our studies of the lactic acid cycle to excised muscle of the diabetic dog. The muscles were removed 4 to 6 days after pancreatectomy. The D:N ratios were used as the index of the completeness of the diabetes. The technic, including the preparation of the muscle strips, was the same as that previously described for the normal animal. The respiratory quotients were determined in parallel sets of Warburg vessels, one containing 0.9 per cent sodium chloride buffered with phosphate to pH 7.4, the other containing in addition m/80 racemic sodium lactate. The changes in lactic acid were determined by chemical analysis of the same muscle strips on which respiratory observations had been made.

RESULTS. *The effect of sodium lactate on oxygen consumption and the respiratory quotient.* The respiratory quotients obtained and the effect of lactate on respiration are summarized in table 1. The diabetic respiratory quotients in saline phosphate averaged 0.73, or close to the theoretical for pure fat oxidation. There is a moderate but definite increase in oxygen consumption (averaging 11 per cent) and a rise in the respiratory quotient (+0.04) of the diabetic muscle in the presence of sodium lactate. Similar indications of the ability of diabetic tissue—kidney and muscle—to oxidize small amounts of sodium lactate have been noted by us before.³

¹ Shorr, E., R. O. Loebel and H. B. Richardson. This Journal, June, 1931, xcvi.

² Boyland, E. Biochem. Zeitschr., 1931, ccxxxvii.

³ Richardson, H. B., E. Shorr and R. O. Loebel. Journ. Biol. Chem., 1930, lxxxvi.

The aerobic oxidative quotient for lactic acid. Here there is a striking difference between normal and diabetic muscle (table 2). In the normal there is a marked disappearance of added lactic acid, 4 mols disappearing for every one oxidized. In the diabetic muscle no such reconversion can be detected chemically. The respiratory quotients indicate moderate but definite oxidation of carbohydrate, presumably the added lactate. It is obvious that it is not a lack of energy from oxidation which is responsible for the failure of resynthesis. Whether the depression of carbohydrate oxidation is sufficient to account for the lack of reconversion is not entirely

TABLE 1

Effect of racemic sodium lactate on oxygen consumption and respiratory quotient of excised normal and diabetic muscle

	RESPIRATORY QUOTIENT		O ₂ CONSUMPTION INCREASE WITH SODIUM LACTATE
	Non-nutrient	With lactate	
			<i>per cent</i>
Normal, 7 experiments	0.94	0.91	+44
Diabetic, 5 experiments	0.73	0.78	+12

TABLE 2

The lactic acid oxidative quotient in the presence of m/80 sodium lactate

	CHANGE IN LACTATE	OXIDATIVE QUOTIENT	RESPIRATORY QUOTIENT
	<i>mgm/gm/hr.</i>		
Normal, 7 experiments.....	-0.6	4.3	0.91
Diabetic, 5 experiments.....	-0.04	1.0	0.78

TABLE 3

Anaerobic formation of lactic acid

	MILLIGRAM OF LACTIC ACID PER GRAM PER HOUR	
	Non-nutrient	0.2 per cent glucose
Normal.....	1.1	1.68
Diabetic.....	0.38	0.36

clear. It remains a possibility, since the failure of resynthesis in diabetes is associated with a profound (even if incomplete) depression of the oxidation of carbohydrate in this disease. Another alternative is a defect in the ferment system which presumably controls the resynthesis of lactic acid.

Anaerobic glycolysis. In diabetic muscle the anaerobic formation of lactic acid was less than a third of normal muscle. In some instances it was almost nil (table 3). Nor was it much increased by the addition of 0.2 per cent glucose. For this one of two explanations may be offered: a diminished supply of precursor, as might be expected as a result of the

lack of synthesis noted above; second, damage to the enzyme system which regulates the synthesis or breakdown of precursor.

SUMMARY. The capacity to resynthesize added lactate is lost in the excised muscle of the completely diabetic dog. There is also a marked reduction in the glycolytic power. Since the lactic acid cycle is so important for the maintenance of the substances concerned with muscular contraction, the failure of this cycle may play a vital part in the failure of the muscular apparatus in this disease.

Some effects produced in vitro by cortico-adrenal extract. HERBERT SILVETTE.

Fresh defibrinated blood from normal dogs was shaken in an incubator at 38 degrees C. together with added glucose and 1, Tyrode's solution; 2, cortico-adrenal extract made by the method of Swingle and Pfiffner, and 3, adrenalin in a concentration equivalent to that found in the extract. All tubes were adjusted to optimum salt content and pH. It was found that at the end of three hours' incubation a fairly constant amount—about 10 to 15 per cent depending on the particular blood used—of the glucose had disappeared from the tubes containing either Tyrode's solution or adrenalin. The decrease in glucose concentration in the tubes containing cortico-adrenal extract was about 50 per cent greater than that observed in the controls. Cortico-adrenal extract is apparently able to increase the rate and amount of glycolysis in the presence of normal erythrocytes. This increased utilization is not due to the minute amounts of adrenalin in the extract. Further experiments are in progress.

Carbohydrate changes in emotion, exercise and exposure to cold. H. SILVETTE and S. W. BRITTON.

The effects of various exhausting motive and emotive conditions on the carbohydrate levels of different animals have been considered. Normal kittens when allowed to swim to the point of exhaustion in a tank of water kept at 37°C. showed considerable muscle and liver glycogen depletion, and concurrent increases in blood sugar and lactic acid. Profound emotional excitation brought about by exposure of cats to a barking dog for a period of several minutes resulted in changes in carbohydrate values quite similar to those observed after severe exercise. On exposure of animals (cats, groundhogs and opossums previously wet with water) in a cold room at about 5°C., a condition of cold narcosis was eventually produced. At this time, reductions were commonly found to occur in blood glucose and lactic acid, as well as in muscle and liver glycogen. In such cases the body temperature and heart rate were also reduced. Maintenance of the blood sugar level was noted to be invariably associated with maintenance of the body temperature within normal limits.

The quantitative relation between concentration of iodo-acetic acid and rate of lactate accumulation in minced muscle. PAUL W. SMITH.

It is found that iodo-acetic acid inhibits lactic acid formation in minced normal muscle in direct proportion to the concentration of the poison. At low concentration the poison only diminishes the rate of lactate accumulation but does not stop it. The findings are interpreted as indicating that the glyoxalase is inhibited by iodo-acetic acid at a wide range of concentra-

tions of the latter but that substantial stoppage of methyl glyoxal mutation occurs only above a critical concentration. This concentration is approximately 0.005 per cent.

The influence of epinephrin upon the exchange of sugar between blood and muscle. SAMUEL SOSKIN, WALTER S. PRIEST and WILLIAM J. SCHUTZ.

The simultaneous determination of the blood-sugar level in the arterial (or capillary) and venous bloods of a limb has recently been used, both in animals and man, as a measure of sugar utilization by the muscles. From his observations that, at a given level of hyperglycemia caused by glucose and epinephrin respectively, the arterio-venous blood-sugar difference is greater in the case of glucose than with epinephrin, Cori has concluded that epinephrin decreases the utilization of sugar by the muscles.

The validity of this interpretation must depend on two assumptions:

1. That the rate of bloodflow through the muscles is the same in both glucose and epinephrin hyperglycemia.
2. That water exchange between blood and muscle remains equal in direction and extent under both conditions.

To test the possible influence of these two factors, we made simultaneous determinations of sugar and total solids of blood from the femoral artery and vein of dogs, while at the same time measuring the venous outflow from the limb.

Our results show that epinephrin, depending partly on the dose, either increases or decreases bloodflow through the muscles; glucose invariably increases the bloodflow. Epinephrin results in a concentration, while glucose almost invariably causes a dilution of the blood passing through the muscles. Both the above assumptions are, therefore, untenable. Our results further indicate that the passage of the blood-sugar into the muscle tissue, under all the conditions we have investigated, is of a reversible and cyclic nature. The corrections for rate of flow and concentration which must be applied to the arterio-venous sugar difference are sometimes sufficient to change an apparent sugar retention into a sugar loss, and vice versa. Our experiments yield no evidence that epinephrin decreases the utilization of blood-sugar by the muscles.

The influence of the previous diet on the respiratory metabolism following glucose meals. HENRY J. SPENCER and WALTER S. McCLELLAN.

Observations have been made in the Sage calorimeter on five normal and five obese subjects both in basal condition and after the ingestion of glucose meals which varied in amounts from 73 to 171 grams. In one series of observations the subjects received liberal amounts of carbohydrate (200 to 450 grams daily) in their previous diet and in the second series they were allowed only 25 grams of carbohydrate daily during the preceding interval. The table contains the average hourly findings for the 2nd to 4th hours after the meal.

When carbohydrate was restricted in the previous diet, the extra heat produced was greater, the amount of glucose oxidized was much less and the apparent storage of glucose as glycogen was greater. Therefore the oxidation of glucose is not the only cause of its specific dynamic action but

	PREVIOUS DIET	
	High carbohydrate	Low carbohydrate
Extra heat production (calories).....	4.8	7.5
Increase in the metabolism (per cent).....	7.2	11.0
Basal non-protein R. Q.....	0.81	0.76
Rise of non-protein R. Q. after glucose.....	0.14	0.07
Glucose metabolized (grams).....	14.5	7.8

the processes associated with the absorption and storage of glucose as glycogen appear to account for it in part if not entirely.

The pyramidal and the extrapyramidal part of the centers of the autonomic system. E. A. SPIEGEL.

The influence of the cortex upon the organs of the vegetative system is mainly due to impulses carried by the pyramidal tract. This is shown by the following facts: The cortical areas from which internal organs can be stimulated are in the close neighborhood of the foci of neighboring skeletal muscles. After cortical injury the fibers of the internal capsule carrying impulses to skeletal muscles, and those carrying impulses to smooth muscles degenerate at the same time (confirmed by Friedberg). Toxins paralyze within the same time the cortical centers for skeletal muscles and those for internal organs (experiments in collaboration with A. Pekelsky). Besides the pyramidal tract, the extrapyramidal system has an influence upon the vegetative segmental centers. One can influence the water metabolism by a puncture of the corpus striatum (Spiegel and Reynolds) and also produce a marked increase of the output of NaCl in the urine by such a puncture (experiments with Dr. Tokay).

Factors influencing the passage of liquids from the stomach into the intestine.

JEAN F. STEWART and W. N. BOLDYREFF.

The factors influencing gastric evacuation with which this paper deals, are grouped under three headings: chemical, mechanical and thermal.

The study of a dog suffering from duodenitis and pancreatitis showed marked delay in gastric emptying for all liquids even tap water.

Strong acids retard the emptying of the stomach. Dilute solutions of them (0.1 per cent and less lactic and 0.05 per cent and less HCl) empty in approximately the same time as does tap water. Alkaline solutions of 1 per cent or more also slow evacuation. Weak basic solutions (of less than 0.1 per cent) act as do the weak acids.

Pepper and mustard infusions and strong solutions (5 per cent) of alcohol produced the most marked retardation in emptying. Quinine, bran, pepsone (10 to 20 per cent), boiled starch (up to 5 per cent) and strong solutions of neutral salts (about 1 or 2 per cent), such as MgSO₄, Na₂SO₄ and NaCl were all slightly inhibitory.

Some of the solutions we tried (for instance, ammonia, NaCl and others) produce gastric secretion and thus causing an acid reaction retard the evacuation of the stomach.

Using 40°C. as the temperature-standard with which all others were

compared, it was observed that gastric evacuation is delayed only by temperatures from 45° to 48°C. and from 5° to 3°C.

Five dogs (four having gastric fistulae and one with intestinal fistula) were used in the 252 experiments carried out. The solutions were introduced into the stomach in 200 cc. quantities usually at 40°C. and were withdrawn at the end of 20 minutes. With some solutions, repeated introductions were made, fractions being withdrawn every 15 or 20 minutes for analysis. The x-ray method was also used in watching the emptying of the stomach.

The most notable factors in inhibiting gastric evacuation are: strong acids and alkalies, strong irritants, high and very low temperatures, pancreatitis, and duodenitis either acute or chronic.

A list of the solutions studied includes the following: HCl, lactic acid, gastric juice, NaHCO_3 , ammonia, MgO , filtered and unfiltered calcined magnesia, lime water, quinine, neutral salts (MgSO_4 , Na_2SO_4 , NaCl), distilled and tap water (very hard), Psyllium seed, bran, mineral oil, pepper, mustard, alcohol, starch and peptone.

The tension-length relation of striated muscle. H. C. STEVENS, E. KARRER and J. SNODGRASS.

The problem investigated is the relation of change of tension to change of length in mammalian muscle (decerebrate cat). Tension is determined by measuring the acceleration given to an inertia disc by the gastrocnemius muscle. Both the angular displacement of the inertia disc and the change of length of the muscle are recorded optically, simultaneously and independently, on a photographic paper. Time in thousandths of a second is recorded on the entire width of the paper by means of a slotted disc driven by a synchronous motor. The rate of travel of the photographic paper is such that the distance between the time lines is approximately 3 mm. By subdividing this space it is possible to read to one-five thousandth of a second. The acceleration of the disc is calculated for each one-thousandth of a second from measurements taken from the record by means of cathetometer reading to one-tenth of a millimeter. The tension exerted by the muscle is calculated from the formula, $\text{Force} = I \times \alpha$ where I is the moment of inertia of the disc and α the angular acceleration. Similar readings for changes in length are taken from the record for each sigma. From the data thus obtained the curves relating tension to change in length and tension to time have been drawn.

Conditioned responses to sound and vibrations. G. F. SUTHERLAND and S. DWORKIN.

Enclosed within a comparatively sound-proof room, dogs were trained to respond to pure musical tones and to tactile vibration. The musical notes were produced by means of a low frequency vacuum tube oscillator, the vibration by means of a vibrating table on which the animals rested. The responses developed were of two types, the secretion of saliva from parotid fistula, and flexion reflex elicited by a strong electric shock.

In the normal adult healthy dogs, responses to air-borne sounds could readily be elicited throughout the frequency range from 50 per second up to 20,000 per second, while one animal responded to a note of 30,000 vibrations. The upper limit of hearing in the dog must therefore be in the neighborhood of the latter figure. Separate responses were then developed to vibratory stimuli, throughout a frequency range from 50 to 300 per

second. Further, one animal was trained to respond positively to vibrations and negatively to air-borne sounds of the same frequency. It is therefore obvious that dogs can distinguish between air-borne sounds and tactile vibrations.

Bilateral surgical lesions were made in the so-called auditory (temporal) cortex, about the fissure of Sylvius. After recovery from acute operative effects had occurred, no clear-cut conditioned reflexes either of a salivary or motor type could be elicited by pure musical tones. To a sound of great intensity a general motor response was evidenced (the head was turned towards the source of sound and the ears pricked up). But this response was absent when a weak note was sounded, though the animal had previously been well conditioned to such a note. These results demonstrate that the general auditory response of Pavlov is essentially a function of subcortical regions and is elicited by a sound of great intensity.

The effect on the albino rat of a ration deficient in inorganic constituents in which edestin serves as the source of protein. PEARL P. SWANSON and ARTHUR H. SMITH.

It has been shown¹ that a retardation of growth occurs accompanied by an active erythropoiesis when the albino rat is maintained for 90 days upon a ration extremely poor in mineral constituents with casein as the source of dietary protein. The red cells are very small in size and the quantity of circulating hemoglobin is reduced. The total blood volume remains normal. On the other hand, when pure edestin replaces the casein in the low salt diet, the characteristic polycythemia is not induced (see table). Instead of finding 12.0 million red cells per cubic millimeter of blood, the normal number, 9.2 million, is present. The cells appear to be only slightly smaller in size than those of rats reared on the edestin ration containing the usual amount of salts. However, the hemoglobin has been reduced to the same low level (13.8 grams per 100 cc.) that characterized the concentra-

The number of erythrocytes, the volume of the red cells and the hemoglobin concentration of the bloods of rats reared on various experimental diets. The average daily intake of water and excretion of urine are shown, also

EXPERIMENTAL DIET	NUM- BER OF RATS	ERYTHROCYTES PER CUBIC MILLIMETER	HEMOGLOBIN PER 100 CC.	RED-CELL VOLUME	INTAKE† OF WATER	OUT- PUT† OF URINE
		millions	grams	per cent	cc.	cc.
Low-ash edestin.	13	9.5 ± 0.19*	13.8 ± 0.19	48 ± 0.33	20 (13 rats)	10
Adequate edes- tin.....	2	9.0	18.9	50	13 (8 rats)	3
Low-ash casein..	26	12.0 ± 0.12	14.3 ± 0.22	45 ± 0.30	13 (12 rats)	4
Adequate casein.	24	9.7 ± 0.11	17.4 ± 0.19	54 ± 0.46	12 (8 rats)	2

* Probable error.

† Preliminary data.

tion of the pigment in the rats that received the low-ash diet in which casein served as the dietary protein (14.3 grams per 100 cc.). The condition resembles the nutritional anemia produced in the rat by the exclusive feeding of a milk diet; in this case, however, the rat is 130 days old.

¹ Smith, A. H. and P. P. Swanson, Amer. Journ. Physiol., 1929, xc, 517.

It cannot be stated at the present time whether the difference in the erythropoietic effects induced by the two inadequate diets is due to a variation in the composition of the small inorganic residues remaining in the respective rations, or whether the intrinsic difference in the proteins incorporated into the low-salt ration is responsible. It has been found that the consumption of the low-salt diet containing edestin causes a consistent diuresis. Rat 344 was observed to drink 80 cc. of water and to excrete 60 cc. of urine in the course of a day. The average daily intake of water and output of urine of the rats on this diet have been found to be 22 cc. and 10 cc., respectively. Normal rats drink approximately 15 cc. of water and excrete 2 to 3 cc. of urine during this interval (see table). The casein ration deficient in salts also tends to be diuretic but not as markedly so as is the similar edestin diet. Neither is the response as regular in all of the rats. The problem is being investigated further.

Observations upon calcium absorption. N. B. TAYLOR and C. B. WELD.

The results of experiments reported in this communication are incompatible with the view that irradiated ergosterol in the dosage which causes an elevation of the serum calcium increases calcium absorption. In the first series of experiments the calcium excretion of dogs was studied for periods of from 10 to 14 days. The calcium excretion in the feces during periods of starvation was no less than when the animals were upon a diet of adequate calcium content. Also when the calcium intake was increased by the addition to the diet of moderate amounts of calcium chloride the calcium output in the feces after the first day or so was no greater than during a preliminary calcium free period. These experiments indicate that an amount of calcium considerably greater than that contained in a normal diet is completely absorbed. Irradiated ergosterol obviously could not increase calcium absorption in these animals. One animal has been studied over a period of seven months, during which time the calcium intake was gradually increased by the administration of calcium chloride. Only a slight increase in calcium excretion occurred when the dose was raised to 1600 mgm. per day. Even when 3200 mgm. of calcium were added to the diet the fecal excretion was increased by only 260 mgm. In this experiment *absorption was quite evidently very greatly increased, yet no significant rise in serum calcium occurred.* In another animal the calcium absorption was increased to nearly 4 grams per day by feeding calcium lactate, yet no rise in serum calcium was observed.

The absorbed calcium is undoubtedly stored within the body—probably in the bones. It is accepted that irradiated ergosterol in overdosage withdraws calcium from the bones. It follows that calcium deposition is also prevented by the sterol. The effect of high calcium intake in encouraging the hypercalcemic effect of irradiated ergosterol is well known. This effect has been assumed by Harris and Innis,¹ Jones and Rapoport,² and others, to be the result of increased absorption, but it is due more probably, in our opinion, to the fact that the large amount of calcium absorbed when the calcium intake is high, being prevented from entering the bones, accumulates in the blood and is added to that withdrawn from the skeleton. The effect of added calcium in enhancing the action of parathormone

¹ Harris and Innis. *Biochem. Journ.* 1931, xxv, 367.

² Jones and Rapoport. *Journ. Biol. Chem.*, 1931, xciii, 153.

which admittedly does not increase absorption may be explained in a similar manner.

In another series of experiments pups were given relatively small doses of irradiated ergosterol (0.3 cc. 250 D per kilo, or about $2\frac{1}{2}$ times the maximal infant dose). Calcium excretion was nearly doubled.

The action of the vagus on the heart in acute anoxemia with further observations on the question of cardiac tonus. EDWARD J. VAN LIERE and GEORGE CRISLER.

The work was done on barbitalized dogs. A tracheal cannula was inserted; a carotid cannula was put in to obtain blood pressure tracings and the vagi were exposed. Anoxemia was produced by allowing the animal to breathe pure nitrogen out of a bag connected with the tracheal cannula. A flutter valve in the system prevented accumulation of carbon dioxide. The animal was kept underneath the x-ray machine during the entire experiment; the distance from the target to the film was one meter. The films were exposed 5 seconds. A control x-ray picture was taken, anoxemia was produced and x-ray pictures were taken at the end of $\frac{3}{4}$, 1, $1\frac{1}{4}$ and $1\frac{1}{2}$ minutes. The animal was allowed to return to normal. The vagi were cut; a control x-ray picture was taken and 4 exposures were made during anoxemia at the same time intervals as was done in the control series. The area of the cardiac silhouettes was measured with a planimeter.

With the vagi intact during anoxemia the hearts grew progressively larger and the cardiac rate progressively decreased. After double vagotomy during anoxemia the heart dilated less and the rate decreased less. Vago-spasm from anoxemia was confirmed by this method. The greater cardiac slowing during anoxemia with the vagi intact probably accounts for the greater dilatation. Animals with greater differences in cardiac rate before and after vagotomy showed relatively greater differences in cardiac size. Since the cardiac rate could easily account for the changes in cardiac size and there is no particular indication of any "tonogenic" influence, this work may be considered further evidence that the vagi have no effect on cardiac tonus in the mammalian heart.

The effect of ultra-violet radiation on the pressor action of nicotine. D. J. VERDA, O. S. ORTH and W. E. BURGE.

The following is a description of a typical experiment performed in an attempt to determine the effect of ultra-violet radiation on the pressor action of nicotine. Fifty cubic centimeters of nicotine diluted 1:1,000 were introduced into a flat bottomed glass dish 8 cm. in diameter. This was placed under the quartz burner at a distance of 15 cm. in running water to keep the solution cool and prevent evaporation during the experiment. The quartz mercury arc was operated at approximately 140 volts and 3.3 amperes. Ten cubic centimeters of solution were removed before the irradiation began to serve for a control. The burner was then started and after 15 minutes 10 cc. more of the solution were removed. Similarly after 30 and 45 minutes of irradiation other 10 cc. portions were removed. Five-tenths of a cubic centimeter per kilogram of body weight of these solutions irradiated for the different periods of time, as well as the non-irradiated solution, were introduced into the external jugular of a dog and blood pressure records were made with the use of a mercury manometer.

Approximately 10 seconds were required for an injection and these were made at 15 minute intervals. The control or non-irradiated nicotine produced a rise in blood pressure of 52 mm. Hg; that irradiated for 15 minutes caused a rise of 30 mm. Hg; that irradiated for 30 minutes caused a rise of 12 mm. Hg, and the nicotine irradiated for 45 minutes had no effect on blood pressure. Several experiments similar to the preceding were carried out with fairly comparable results.

On the establishment of a membrane equilibrium in the dynamic ultrafiltration process. MAURICE B. VISSCHER and RAYMOND C. INGRAHAM.

There has been doubt as to whether, during the active filtration process, the Donnan membrane equilibrium can be set up, the argument being that adequate time is not allowed for a diffusion equilibrium to be set up. Experiment shows that ultrafiltrates from plasma show Na and Cl concentrations differing from those in plasma in the direction predicted by the Donnan equation. The ratio r , however, is consistently lower for Na than for Cl. Assuming that the activity of Cl in plasma is not different from its activity in similar non-protein solution, it might be concluded that the activity coefficient for Na is depressed in protein solutions. There is collateral evidence to support this view.

Decreasing the base bound by protein by increasing the CO₂ tension invariably raises the r ratio for both Na and Cl, as is predicted if the phenomenon follows the Donnan law.

Calculation from Fick's diffusion law shows that one might expect diffusion equilibrium to be reached in a system like that employed.

Contraction and evacuation of the gall bladder of the rabbit produced by cholecystokinin. E. L. WALSH.

The gall bladder of the rabbit has been seen by direct vision to contract and evacuate via the cystic duct under the influence of cholecystokinin. Photographs have been made. Similar evidence of evacuation of the viscus has been obtained by analyzing the common-duct bile for methylene blue (15 hours after administration) before and after the injection of cholecystokinin. The gall bladder of the rabbit does not contract with sufficient force to evacuate the larger portion of a viscous brominated oil placed within its lumen artificially, which is known to be evacuated in the cat and dog.

The osmotic pressure of the colloids of lymph from the lacteals as a measure of the absorbing force of the intestine. HERBERT S. WELLS.

The osmotic pressure of the proteins of lymph from the lacteals is found to correspond closely to the absorbing force of the intestine as measured by the negative intra-intestinal pressure necessary to abolish the absorption of fluid from isolated jejunal loops of anesthetized dogs. In the course of the 14 experiments of the series determinations were made of the osmotic pressure of colloids of blood serum and of lymph from the lacteals; of the total protein, albumin, and globulin content of lymph and of serum; of the mesenteric venous pressure; and of the absorbing force.

Analysis of the findings seems to justify the assumption that, for the case of the intestine, the protein concentration of the lymph is identical with that of the tissue fluids of the villi. It follows that the absorbing force of the intestine is due to the osmotic pressure, exerted against the

semipermeable epithelial membrane of the intestine, of that portion of the total serum proteins to which, on the average, the walls of the capillaries of the villus are permeable.

The data indicate further that the protein content of the lymph is restricted to the range of from $\frac{1}{3}$ to $\frac{2}{3}$ of the protein concentration of the serum. The value of the osmotic pressure for 1 per cent protein of the lymph being approximately the same as that of the same concentration of the serum protein, it follows that the magnitude of the absorbing force of the intestine may not be less than $\frac{1}{3}$ nor greater than $\frac{2}{3}$ of the osmotic pressure of the serum proteins. The absorbing force, consequently, may be considered to be regulated by those processes of the body which govern the concentration of the blood proteins; in other words, the energy for the absorption of fluid is provided, under normal conditions, by the energy expended in the excretion of water from the body.

The molecular concentration of glomerular fluid. H. L. WHITE.

The molecular concentration of glomerular fluid has been compared (Barger's method) with that of serum in 84 experiments on necturus and 6 on the frog over a period of $2\frac{1}{2}$ years. In 5 of the frog experiments concentrations were equal, in 1 the glomerular fluid was apparently less concentrated; in none of these was the fluid transferred from pipette to capillary inside a moist chamber. The necturus experiments may be divided into 3 groups, those where the transfer was made without a moist chamber, those with a relatively inefficient moist chamber, and those where the air was satisfactorily saturated with water vapor. In the first group of 48 experiments the concentration of the glomerular fluid was much greater than that of the serum in 11, somewhat greater in 12, the same in 15 and less in 2; 8 were rejected because of variations in controls. In the second group of 15 experiments the concentration of glomerular fluid was much greater than that of the serum in 6, somewhat greater in 3 and the same in 2; 4 were rejected because of variations in controls. In the third group of 21 experiments the concentration of glomerular fluid was much greater than that of the serum in 2 (collection from 2 capsules in 1 case), somewhat greater in 5 (2 of these doubtful), the same in 13 and less in 1. Since various modifications of technique, seasonal variations in animals, etc., arose throughout the course of the work the interpretation is not simple, but probably the most important single variation is in the technique of transference. Contamination by tubular fluid has been excluded. My present impression is that the glomerular membrane is probably a passive filter.

The rôle of the spleen in iron metabolism. ELIZABETH D. WILSON and E. B. KRUMBHAAR.

Previous work in this school and elsewhere having shown discordant results in iron elimination in dogs after splenectomy, the work has been repeated with a larger number of animals and a longer series of intact and operated controls, all on a synthetic diet (Cowgill-Karr) of known iron content. The complete blood picture has been followed throughout. As in previous studies a transient post-splenectomy anemia was observed. Six dogs were splenectomized. Each animal had an adequate preoperative control period. For further control two intact animals were followed (one for a year and a half); five animals were studied before and after

major operations other than splenectomy and two during development and recovery from a severe anemia (pyrodin). In all animals studied, considerable variation in iron balance was observed in the periods used (6-14 days). In general we have found loss of weight to be accompanied by a negative and gain of weight by a positive balance. The intact controls had positive balances, the one followed for a year and a half having over 0.4 mgm. per kilo per day, except when fasting or on an inadequate intake when output nearly equalled or exceeded intake. Of five animals subjected to major operations other than splenectomy, all of which in preoperative periods had positive balances varying between 0.423 mgm. to 0.075 mgm. per kilo per day, three showed a positive balance of from 0.989 mgm. to 0.057 mgm. and two showed negative balances of from 0.02 to 0.18 mgm., the last two developing anemia while the others did not. Two dogs with a severe anemia (pyrodin) had negative balances of 0.739 mgm. and 1.09 mgm. respectively during the period of development of the anemia; both had positive balances of 0.401 and 0.476 mgm. respectively during recovery. Of the six splenectomized dogs, five showed a greater tendency to loss of iron after splenectomy (i.e., either a change from a positive to a negative balance, or to an increasingly negative balance). This was not always apparent until some days after splenectomy, and coincided approximately with the period of developing anemia. The sixth, for reasons that are not apparent, showed an even greater positive balance in the postoperative periods, but it was not possible to study the period of developing anemia. Removal of the spleen was therefore usually found to be transiently associated with increased excretion of iron. It is not possible to demonstrate, however, that the increased loss of iron is the cause of the anemia.

Peripheral and central chemical control of respiration. CLAUDE V. WINDER, HARRIET OWEN and ROBERT GESELL.

Respiratory stimulation from cyanide injection into a carotid artery, reported by Heymans and others,¹ and Owen and Gesell,² is consistently demonstrable. The former conclude that the stimulating action of cyanide is due exclusively to reflexes from the carotid and cardio-aortic zones.

In view, however, of effects of cyanide and sulphide injection into the fourth ventricle, an attempt is being made to determine the relative importance of both central and peripheral cyanide effects.

Usually, injections of 0.5 to 1.5 mgm. of sodium cyanide into a denervated carotid, with the external carotid occluded, gave no effects that were not attributable to incomplete denervation. In one animal a slight depression was observed. Occasionally delayed stimulations, sometimes marked, where both sinuses were certainly denervated, resulted. There were similar occasional stimulations after double vagotomy. Variations in the anastomosis of the internal carotid with the medullary circulation are borne in mind in connection with these variable results. The effects of vertebral artery injections with patent carotids or with double sinus denervation did not, as groups, differ considerably. They were very

¹ Heymans, C. P., J. J. Bouchaert et L. Dautrebande. Arch. Int. de Pharmacod et de Thérap., xl, 54.

² Owen and Gesell. Proc. Soc. for Exp. Biol. and Med., xxviii, 765.

occasionally depressions, but usually stimulation, or primary stimulation with secondary depression to or below normal, and frequently tertiary persistent increased amplitude.

When the carotid innervation had not been damaged, clamping of the common carotid, or complete vascular isolation of the sinus, reduced the vertebral dose required to stimulate often to below that required directly in the normal carotid, and sometimes to that required in the innervated carotid with its external branch occluded; and reduced the latency frequently to that of the carotid effect. Clamp pressure on the carotid body and nerves removed the vertebral effect back to the slow, less intense, and variable one. In one dog, where common carotid occlusion did not occasion the usual intense vertebral effect, it was readily demonstrated after double vagotomy.

Further work is necessary to definitely base these intense vertebral effects on either an action of cyanide at the center, as an augmentation of peripheral effects possibly from the non-pulsating or less turgid sinus; or on some remarkably freely attained reflex zone innervated through the carotid plexus.

The diffusion of carbon dioxide through tissues. C. I. WRIGHT.

Membranes of animal tissues were placed between an atmosphere of known carbon dioxide tension and a barium hydroxide solution. The rate at which the carbon dioxide diffused through these membranes was determined by following the conductivity changes of the barium hydroxide solution (Fenn 1928).

The diffusion rates through several types of membranes are summarized in the following table:

MEMBRANE	NUMBER OF DETERMINATIONS	PERMEABILITY CONSTANT
Frog muscle.....	23	$4.3-6.0 \times 10^{-4}$
Mammalian muscle.....	1	4.7
Mammalian smooth muscle.....	1	4.4
Mammalian connective tissue.....	2	2.7
Frog skin.....	24	2.8-3.3
Frog skin (acidified $\frac{n}{10}$ HCl).....	3	4.5
Rubber.....	3	0.48

Temperature 22°C.

Permeability is expressed in cubic centimeters per square centimeter under a pressure gradient of one atmosphere per centimeter.

Skin and connective tissue offer a much greater resistance to the diffusion of carbon dioxide than muscle. Acidification of frog skin increases the permeability more than would be expected from the change in water content. These differences in permeability are probably due to differences in structure. All the tissues studied have a permeability constant for carbon dioxide less than that of water (approximately 8.2×10 at 22°C.).

That bicarbonate contributes to the total diffusion of carbon dioxide is shown by an increase in the permeability constant when low experimental tensions of carbon dioxide are used. Since this is so, a true diffusion constant ($K = \text{cm}^2/\text{min.}$) cannot be expressed until the amount contributed by the bicarbonate has been evaluated.

Reversal of the vascular response to a small dose of adrenalin in the rat.

LELAND C. WYMAN and CAROLINE TUM SUDEN.

It has been stated that no depressor responses to intravenous injections of adrenalin occur in the rat. This was confirmed in urethanized rats, minimal effective doses (0.05 cc. of 1:2,000,000) producing purely pressor effects. In etherized rats the smallest effective doses sometimes produced depressor responses. Larger doses always produced pressor responses. Adequate control injections of saline and of chloretone solutions were given.

In suprarenalectomized rats having autoplasmic transplants of cortical tissue but no demonstrable chromaffin tissue, and exhibiting normal blood pressures under urethane anesthesia, depressor responses are regularly obtained with small doses of adrenalin. Larger doses produced pressor-depressor responses and much larger doses were required to produce purely pressor effects.

In normal rats, either urethanized or etherized, a blank operation or single suprarenalectomy did not alter the vascular responses to adrenalin, given from 2 to 73 minutes after operation. Removal or simple ligation of both suprarenals, with no accompanying alteration of blood pressure, always reversed the vascular responses to small doses. Doses which had previously produced pressor responses now gave depressor responses, and in etherized animals the depressor responses to small doses were greatly increased. As much adrenalin was required to produce pressor effects as in the case of the transplanted rats. In several cases the normal control, the control operation and ligation of the suprarenals were all carried out in succession on the same animal.

No significant changes in heart rate were observed. Microscopic observation of blood vessels during the injection of small doses of adrenalin in normal and in transplanted rats, showed only constriction of the ear vessels, predominating constriction of the intestinal vessels in normals, and brief constriction followed by marked dilatation of intestinal vessels in transplants. The depressor phenomenon induced by lack of the suprarenal medulla is evidently of vascular origin.

CHANGES IN THE BLOOD ASSOCIATED WITH FEVER INDUCED BY KILLED *B. COLI*

M. GARCIA BANUS AND EMANUEL GINSBURG

From The Department of Physiology, Tufts College Medical School, Boston

Received for publication November 20, 1931

In a previous paper by one of us (Banus, 1929) certain changes in the blood of dogs subjected to physical hyperthermia were described. In these experiments emphasis was laid on the changes occurring during the prolonged maintenance of the temperature at a high level, rather than on those resulting from the initial increase in temperature. This was done in order to afford a more valid comparison with clinical cases suffering from prolonged high temperature, i.e., fever. The changes observed were an increase in the concentration of the blood accompanied by a decrease in the O_2 capacity, a lowering of the CO_2 dissociation curve and especially a change in its slope, the latter being dependent upon an increase in the buffering power of the blood.

It seemed desirable to make similar observations in hyperthermia of a type more closely related to clinical fevers.

The blood of patients suffering from febrile conditions has been frequently studied. In pneumonia normal or somewhat lowered CO_2 dissociation curves have been described by Means, Bock and Woodwell (1921) and by Barach, Means and Woodwell (1922). An alkaline reserve lower than normal has been repeatedly found in clinical fevers as well as in the cases of physical hyperthermia. Yamakita (1921) observed a lowering of the O_2 dissociation curve which he attributed to an increased acidity of the blood. No change in the slope of the CO_2 dissociation curve or in the buffering power of the blood has to our knowledge been described in febrile cases.

To determine whether the changes observed in physical hyperthermia occurred also in hyperthermia of a type similar to clinical fevers, a high body temperature was induced in dogs by intravenous injections of killed *B. coli*. Barbour (1926) has studied the changes in the blood produced by fever due to subcutaneous injection of *B. coli* vaccines, but he was principally interested in alterations occurring in the blood only while the temperature was changing. As soon as the maximum temperature was obtained, he produced antipyresis.

In the present series of experiments on the effect of fever produced by

B. coli we have maintained the body temperature of the dogs at several degrees above normal for many hours and have studied the effect on the blood of this prolonged hyperthermia.

METHODS. Fever was induced by intravenous injections of heat-killed *B. coli* suspensions obtained from 14 to 24 hour broth cultures.¹ As, according to Vaughan and Vaughan (1913), a series of injections of increasing strength is more effective in producing high temperature than is a single large one, several injections, starting with 4 to 5 cc. and increasing progressively to 8 or 10 cc., were given, with two-hour intervals between injections. (In preliminary experiments subcutaneous injections of milk and Lilly's typhoid and *B. coli* vaccine had been given without obtaining a sufficiently prolonged fever.)

Rectal temperature was obtained every hour at the beginning of the experiment and at longer intervals when the maximum change in temperature had occurred.

Blood was obtained from the jugular vein, clotting being prevented by the addition of 2 drops of a 20 per cent solution of heparin for each 5 cc. of blood drawn. One sample of blood, obtained before the first injection of *B. coli* suspension, was named sample I in all experiments and used as a control. A second and a third sample were obtained during the period of high temperature, usually 4 to 6 hours and 8 to 11 hours respectively after the first injection. In the later experiments a fourth sample was obtained the next day, 24 to 30 hours after the first injection, when the temperature had returned to normal. Great care was taken to avoid the excitement anhydremia of which Barbour speaks, the dogs being trained to submit to the drawing of blood.

In each of the samples the following factors were determined:

1. The concentration of the blood, using the total solids as an index. This was determined gravimetrically by Van Slyke's method. The average error was ± 0.09 , the maximum error ± 0.18 gram per 100 grams of blood.

2. The oxygen capacity of the blood after saturation with air at room temperature, determined by the method of Van Slyke and Neill. The average error was ± 0.09 volume per cent; the maximum, ± 0.3 volume per cent. Dividing the oxygen capacity by Hufner's figure (1.34), the hemoglobin concentration was obtained.

3. The CO_2 dissociation curve, obtained by the determination of the CO_2 combining power of the blood at three CO_2 tensions. Saturation of the blood was effected in a thermostat at 38.0°C ., according to the method described elsewhere (Banus, 1929). Mixtures of air and CO_2 at CO_2 tensions of 10 mm., 30 mm., and 50 mm., were used to obtain the three points on the curve. The CO_2 combining power of the blood at these three CO_2 tensions

¹ The cultures were kindly prepared by Dr. Frederick Parker, of the Pathological Laboratory at Boston City Hospital, to whom we wish to express our indebtedness.

was determined by the Van Slyke-Neill method. The average error was ± 0.27 volume per cent, the maximum error ± 0.5 volume per cent.

4. The alkaline reserve of the blood (i.e., the CO_2 combining power at 40 mm. CO_2 tension) was interpolated from the CO_2 dissociation curve.

5. The total pigment obtained in order to detect the formation of methemoglobin or other non oxygen-carrying pigment was determined by Stadie's method (1920). The average error was ± 0.12 gram of pigment per 100 cc. of blood.

No determinations of the blood pH were made in this series of experiments, therefore the buffering power of the blood could not be calculated. But the work of Peters (1923) indicates that changes in the blood buffering power are directly related to changes in the slope of the CO_2 dissociation curve. This slope, in turn, can be expressed in terms of the increment of the CO_2 combining power between two different CO_2 tensions. We have employed the values at 10 and 50 mm. CO_2 tension in this connection.

RESULTS. *General effects.* One hour after an intravenous injection of *B. coli* suspension the temperature of the dog began to rise. This was accompanied by shivering and increase in muscle tonus as observed by Barbour. A second injection after two hours produced a further rise in temperature. The effect of a third injection (even of a larger dose) was not so marked.

The temperature attained a maximum from 3 to 5 hours after the first injection. After the peak was reached a high level was maintained for 4 to 5 hours more; the temperature then gradually returned to normal. In some cases the temperature was still above normal after 24 hours. A fourth and fifth injection (at, respectively, 6 and 8 hours after the first) had no apparent effect upon the slope of the temperature curve. A maximum temperature of 41.5°C . or about 3°C . above the normal was obtained in one case with complete recovery of the dog. In general the maximum was between 40 and 41°C .

The change in temperature was accompanied by symptoms indicating toxic effects. After the first and second injections there frequently was vomiting. When large doses had been given, intestinal disturbances appeared in the form of frequent stools and even diarrhea. Four of the dogs died within 48 hours, death being preceded, in two of these, by bloody stools. In the remaining cases the animals recovered.

In the two experiments where subcutaneous injections were used, the temperature did not start to rise until after two hours, when two injections had already been given. The rise was more gradual, the peak being only reached after eight hours, and was not so high as in the case of intravenous injections. A maximum temperature of 40.4°C ., or two degrees above the normal, was reached after four injections. A third and even a fourth injection definitely contributed to the final rise in temperature. After the peak the temperature dropped gradually without an intervening plateau.

TABLE 1
Effect of fever induced by *B. coli* on certain blood factors

EXPERIMENT NUMBER	BLOOD SAMPLES		TOTAL SOLIDS		O ₂ CAPACITY	HEMOGLOBIN			INACTIVE PIGMENT	ALKALINE RESERVE	Δ [CO ₂] (15-50 MM. p CO ₂)	HOURS OF FEVER ABOVE 39°C.
	Number	Time after 1st injection	Concentration	Change from normal		Calculated from O ₂ capacity	Change from normal	Calculated from total pigment				
			A	B								
		hours	per cent	per cent	vols. per cent	grams per 100 cc.	per cent	grams per 100 cc.	grams per 100 cc.	vols. per cent CO ₂	vols. per cent	
7	I	0	18.35		16.0	11.9		13.1	1.2	45	21	0
	II	4½	20.65	12.5	21.0	15.7	31.2	16.8	1.1	35	21	3½
	III	9	21.93	19.5	22.0	16.4	37.5	18.4	2.0	42	24	8
8	I	0	19.71		19.5	14.6		14.8	0.2	49	23	0
	II	6	21.02	6.6	22.9	17.1	17.4	16.8	-0.3	33	21	4
	III	11½	23.05	16.9	23.4	17.5	20.0	20.0	2.5	40	23	6
	IV	30	22.70	15.2	22.8	17.0	16.9	19.4	2.4	35	32	4
9	I	0	19.15		18.3	13.7		13.7	0.0	45	21	0
	II	5½	21.40	11.7	22.4	16.7	22.4	17.1	0.4	40	23	4
	III	12	20.67	7.9	21.2	15.8	15.8	16.4	0.6	40	22	10½
	IV	29	18.88	-1.4	19.0	14.2	3.8	14.1	-0.1	44	21	16
12	I	0	21.52		22.3	16.6				47	24	0
	II	5	24.29	12.9	28.0	20.9	25.6			40	25	4
	III	10	25.54	18.7	28.2	21.0	26.4			40	27	9
	IV	24	23.60	9.7	24.0	17.9	7.6			40	23	13
13	I	0	17.95		16.5	12.3				47	18	0
	II	6	20.74	15.5	20.9	15.6	26.7			33	20	5½
	III	13	19.07	6.2	17.9	13.4	8.5			39	23	9
	IV	25	17.88	-0.4	15.9	11.9	-3.6			45	23	9

Experiments ending with death of animal

5*	I	0	20.4		20.8	15.5		15.5	0.0	43	21	0
	II	3	24.8	21.6	26.6	19.8	27.9	21.2	1.4	17	13	2
10**	I	0	19.88		19.4	14.5		14.5	0.0	51	22	0
	II	6½	22.64	13.9	25.5	19.0	31.4	18.9	-0.1	30	20	5
	III	11	23.18	16.6	24.5	18.3	26.3	18.9	0.6	25	18	9½

* Dog died while sample II was being taken.

** Dog died 30 minutes after sample III.

Changes in the blood. The changes observed in the factors studied are summarized in table 1.

1. *Changes in blood concentration.* A marked increase in the concentration of total solids in the blood was observed in all cases (table 1, columns A and B). This change was very rapid during the first few hours, reaching a value of 10 to 20 per cent above normal in the 4 to 6 hour sample. The maximum value usually occurred later (expts. 7, 8, 9 and 12), this further increase occurring when the peak of the fever had passed and the temperature was gradually falling. In only two cases (expts. 9 and 13) was a falling temperature associated with decreasing concentration when the third sample was taken. The blood concentration gradually returned toward the normal value, the increase, however, persisting long after the temperature had reached the normal.

2. *Changes in O₂ capacity and hemoglobin concentration.* (Columns C; D and E.) With such marked changes in blood concentration one would expect changes in the O₂ capacity and hemoglobin concentration. But the interesting point is that these changes are not parallel to the changes in blood concentration. During the first hours the change in O₂ capacity was always much greater than the increase in blood concentration (compare columns B and E). Later these relative rates of change are reversed: the concentration increased more rapidly than did the O₂ capacity (expt. 8, 12); the O₂ capacity was even decreasing while the blood concentration was still increasing (expt. 10); or if, as occurred in experiment 13, the concentration had started to decrease, the O₂ capacity fell even more rapidly. The only exception to this was experiment 9, in which case only two small injections having been given, the dog's reaction was smaller than that of any other observed. Later, during the period of recovery, the hemoglobin concentration in all cases returned towards normal at a greater rate than the blood concentration.

3. *Changes in alkaline reserve.* There was in all cases (column H) a definite drop in the alkaline reserve, not proportional to the increase in temperature. This differs from the findings in physical hyperthermia by Walinski (1929), who observed such a proportionality. In all the cases not ended by death there was a tendency later to return to normal, this return being practically complete within 24-30 hours in two out of the four cases studied that long.

The fall in the alkaline reserve noted in these experiments agrees with many observations in clinical cases, for example, those of Means, Bock and Woodwell (1921), Barach, Means and Woodwell (1922) and Bruhl (1929); as well as with the results obtained in experimental fevers in dogs by Leake, Vickers and Brown (1924) and by Barbour (1926). It also agrees with the results in physical hyperthermia obtained by Banus (1929) and, except in the proportionality to temperature, with those of Walinski (1929). Where, in clinical fevers or hyperthermia cases, no fall of the alkaline reserve has been noted, this is probably due to the fact that the fever was not high or prolonged enough to produce the effect.

4. *Changes in the CO₂ dissociation curve.* Changes in both the height and the slope were observed. Upon the former depend the changes in alkaline reserve which were discussed in the preceding paragraph. The change in slope is indicated by the increase in the increment of the CO₂ combining power between 10 and 50 mm. CO₂ tension (table 1, column I). This increase, although small in most of the cases, was outside our limits of error. It varied from three to nine volumes per cent CO₂.

In contradistinction to the change in height of the curve, which occurs in the earlier samples, the change in slope did not occur until several hours after the first injection of vaccine. The time when the slope was greatest corresponded to the period of maximum blood concentration and O₂ capacity in only about one half the cases. In the other cases it occurred when the latter values were already returning to normal.

Although no calculation of the buffering value of the blood can be made as there were no pH determinations, the increase in the slope of the CO₂ curve is indicative of an increase in the buffering power of the blood for CO₂.

5. *Changes in total pigment.* In the previous experiments with physical hyperthermia (Banus, 1929) a drop in the O₂ capacity concomitant with an increase in the blood concentration was observed, and was interpreted as possibly indicating a conversion of the hemoglobin into a non oxygen-carrying substance. In this series of experiments these changes were of a different character, as has been discussed under 2. The fact, however, that in the later hours the O₂ capacity was diminishing more rapidly than the blood concentration, seems to confirm the possibility of the conversion. Further evidence may be obtained from a comparison of the hemoglobin as calculated from the O₂ capacity, and that determined from the total pigment (columns D and F). In five cases out of the seven studied there was at the end of the experiment a definite discrepancy between these two values (column G). This discrepancy might be interpreted as due to the presence of a non-functional pigment, the nature of which was not determined.²

Changes occurring just before death. In one case (expt. 5) a sample of blood was obtained just as the animal died; in another (expt. 10) about half an hour before death. In both cases the following changes were observed: A very great increase in the concentration of the blood. In case 5 it rose 21.6 per cent above normal, which was the maximum change observed. At the same time there was present a very marked increase in

² Since this work was completed a paper by Haurowitz and Reiss has appeared (Zeitschr. f. physiol. chem., 1931, cxviii, 191). These investigators claim that the discrepancy between the hemoglobin values obtained by means of the oxygen capacity method and those obtained by the colorimetric method in splenectomized dogs is not due to the appearance of inactive pigment but is an artifact due to the presence of a lipid in the plasma. Whether this observation has any bearing on the discrepancy found by us in fever cases is being examined in similar experiments now in progress.

fixed acid in the blood as determined by the extreme lowering of the alkaline reserve. The increased amount of acid must have removed a large amount of the available base of the blood as the buffering power was diminished. The slope of the CO_2 curve was decreased. The increment between 10 and 50 mm. CO_2 tension was reduced to 13 and 18 volumes per cent as against the average normal value of 22. The non-functional pigment was no greater than in many of the other experiments.

DISCUSSION. Our results, first of all, indicate that certain definite differences exist between physical hyperthermia and that induced by the injection of killed *B. coli*. In both cases, it is true, there is an increase in blood concentration, a fall in the alkaline reserve, and a change in the slope of the CO_2 dissociation curve. But the changes following the injection of *B. coli* were not proportional to the height of the fever, whereas in physical hyperthermia the indications are that such a proportionality exists. Moreover, in the present experiment, the blood changes frequently outlasted the fever. For example, in four out of eight cases where more than two samples were studied, the blood concentration was still increasing when the temperature was falling. This is at variance with the results obtained by Barbour with induced antipyresis.

Barbour in his experiments, observed that with a rising temperature, there was an increased red cell count and an increased concentration of whole blood, with no appreciable change in plasma concentration—which may be expressed simply as an increased number of *R.B.C.s* per unit volume of blood. This he interpreted as due to escape of plasma from the blood. In the early hours of our experiments the fact that the increased O_2 capacity and hemoglobin concentration were much greater than the corresponding increase in blood concentration would also point to an increase in the *R.B.C.* count per unit volume of blood—in other words, our results in these hours agree with those of Barbour. However, while his interpretation cannot be ruled out, the observed facts might also be explained by the addition of red cells to the blood, for example, by contraction of the spleen as suggested by Barcroft (1922, 1925). Neither Barbour's experiments nor ours throw light on this matter.

In the later hours, after prolonged duration of fever, new factors seem to appear or become dominant. This is indicated by the following facts: 1. There is a reversal of the previous increases in hemoglobin and blood concentration. 2. The hemoglobin concentration is usually diminished more rapidly than is the blood concentration.

There has been in addition, during the later hours, an increase in the buffering power of the blood, as shown by a change in the slope of the CO_2 dissociation curve. This increased buffering capacity is doubtless due in part to the increased blood concentration, but is certainly not entirely explained by it, for in some cases the buffering power is increasing while the blood concentration is falling.

Incidentally, and whatever may be the meaning of the change in slope of the CO₂ dissociation curve, it is clear that in febrile conditions it is unsafe to attempt the establishment of the acid-base equilibrium of the blood by the determination of the CO₂ combining power or the pH at one CO₂ tension only. An increase of CO₂ content at a given CO₂ tension may be obtained even when there is an increase in the fixed acids of the blood.

SUMMARY AND CONCLUSIONS

1. High body temperatures lasting for more than 10 hours were produced in dogs by subcutaneous injections of typhoid and *B. coli* vaccines and by intravenous injections of killed *B. coli* suspensions. The blood was studied during and following the fever for the following factors: Concentration of total solids, O₂ capacity and hemoglobin concentration, CO₂ dissociation curve, and the presence of non-functional hemoglobin pigment.

2. When subcutaneous injections are given the temperature rises slowly, increases with each of every four successive injections, comes to a peak after about 8 hours and drops quickly thereafter. When intravenous injections are given the temperature rises quickly, a maximum being reached after 3 to 5 hours, and is not influenced by any further injection. It remains high for several hours and then drops slowly.

3. The blood changes observed are not entirely due to the effect of temperature but seem to be complicated by other factors.

4. The following changes in the blood were noted:

a. A marked increase in blood concentration. This increase continues after the temperature has begun to drop.

b. An increase in the O₂ capacity and in the hemoglobin concentration. These increases are at the beginning greater than can be accounted for by an increase in blood concentration. After the peak is reached the fall of O₂ capacity and hemoglobin is also greater than can be accounted for by the corresponding fall in blood concentration.

c. A decrease in the alkaline reserve of the blood as shown by a fall in the CO₂ dissociation curve and an increase in the buffering power of the blood as indicated by an increase in the slope of the curve.

5. Death is preceded by a very marked increase in blood concentration and in fixed acids.

BIBLIOGRAPHY

- BANUS, M. G. 1929. This Journal, lxxxviii, 709.
BARACH, A. L., J. H. MEANS AND M. N. WOODWELL. 1922. Journ. Biol. Chem., 1, 413.
BARBOUR, H. G. 1926. Journ. Pharm. Exp. Therap., xxix, 427.
BARCROFT, J. 1925. The Lancet, ccviii, 319.
BARCROFT, J., J. C. MEAKINS, H. W. DAVIS, J. M. D. SCOTT AND W. J. FETTER. 1922. Phil. Trans. Royal. Soc., ccix, 455.

- BRUHL, H. 1928. *Zeitschr. f. d. gesamt. exp. Med.*, lxii, 525.
- LEAKE, C. D., J. H. VICKERS AND F. K. BROWN. 1924. *Journ. Exp. Med.*, xxxix, 393.
- MEANS, J. H., A. V. BOCK AND M. N. WOODWELL. 1921. *Journ. Exp. Med.*, xxxiii, 201.
- PETERS, J. P., JR. 1923. *Journ. Biol. Chem.*, lvi, 745.
- STADIE, W. C. 1920. *Journ. Biol. Chem.*, xli, 237.
- VAUGHAM, V. C. AND J. W. VAUGHAM. 1913. *Protein split products*. Lea & Febiger, Philadelphia.
- WALINSKI, F. 1928. *Deutsch. med. Wochenschr.*, liv, 1831.
- YAMAKITA, M. 1921. *Tohoku Journ. Exp. Med.*, ii, 290.

STUDIES IN THE PHYSIOLOGY OF VITAMINS

XVIII. MEASUREMENTS OF THE VITAMIN B REQUIREMENT IN SEVERAL SPECIES OF ANIMALS¹

GEORGE R. COWGILL

With the collaboration of H. J. DEUEL, JR., ARTHUR H. SMITH, BENJAMIN H. KLOTZ
AND H. H. BEARD

*From the Laboratory of Physiological Chemistry, Yale University, New Haven,
Connecticut*

Received for publication November 23, 1931

The demonstration that the antineuritic vitamin B is required not only by experimental animals of different species but by man as well at once raises important questions. How much of this dietary essential is needed in terms of given available food sources? What are the conditions or factors that determine what the requirement shall be in any given instance? The value of knowing the answers to these questions, particularly with reference to the human being, requires no extended argument. The proper mode of approach to these answers is by no means obvious. The problem is well-nigh insoluble if one is forced to rely on measurements made on the human species itself. A plan, offering some promise of success, was tried out in the research to be reported in this paper. A given vitamin-containing material was tested quantitatively on several species, namely, the mouse, rat, pigeon and dog. The minimum amount of this product required by each of a group of individuals of each species was determined. The several groups of data were then studied and compared with the hope that certain underlying fundamental relationships would be revealed which would suggest one or more of the factors that determine the organism's requirement for this vitamin.

From reports in the literature it is evident that the vitamin B requirement stands in some relation to body weight. For example, what suffices for a 100 gram rat (Osborne and Mendel, 1922) is quite inadequate for a

¹ The experiments reported in this paper were begun in 1922. Reports of the progress of this research have been presented from time to time as follows: G. R. Cowgill and H. J. Deuel, Jr., 1923, Proc. XIth Internat. Physiol. Congress, Edinburgh, p. 91; G. R. Cowgill, A. H. Smith and H. H. Beard, 1925, Journ. Biol. Chem., lxxiii, p. 23; G. R. Cowgill and B. H. Klotz, 1927, This Journal, lxxxi, 470; G. R. Cowgill, H. A. Rosenberg and J. Rogoff, 1930, This Journal, xciii, 641; E. Burack, and G. R. Cowgill, 1931, Proc. Soc. Exper. Biol. Med., xxviii, 750.

much larger animal. Possibilities in the way of a closer approximation to an organism's requirement for vitamin B than in terms of body weight alone were suggested by a comparison of our data pertaining to the rat and dog. In 1923, when there were reported from this laboratory the results of experiments on the requirements of white rats and dogs of what was then called vitamin B in the form of the *same* yeast concentrate (Cowgill and Deuel, 1923), our curiosity was especially aroused by the fact that, on the basis of these experiments, the rat was found to require approximately five times as much vitamin B as the dog per unit of body weight. Inasmuch as the basal metabolic levels of these two species stand in a somewhat similar relation to each other, there was suggested the possibility that the vitamin B requirement may be related in some way to metabolism, perhaps the number of calories metabolized, or the surface area, "active protoplasmic mass," or some other metabolic entity. Inasmuch as such factors stand in certain fairly well-defined relationships to body weight, the published observations of Osborne and Mendel and others that might be mentioned, indicating some relation between vitamin requirement and body weight, can be regarded as confirming such an idea. The importance of an experimental investigation of such a possibility will be further appreciated when it is realized that the establishment of some general relationship in several species paves the way to a determination of man's requirement for this vitamin, and perhaps the need for other dietary essentials as well. The late Dr. T. B. Osborne² once remarked that "man cannot possibly require relatively as much vitamin B as rat experiments would suggest." If the rate of metabolism or some function of it is involved in the determination of an organism's requirement, and an expression valid for several species is arrived at, it should become possible to interpret properly for man's nutrition quantitative experiments performed on rats and other animals. In this paper are presented the results of experiments bearing on this question performed in this laboratory on dogs, pigeons, rats and mice. The *same* vitamin-containing material was used in *all* of these experiments, namely lot 985 of Yeast Vitamine Powder (Harris).³

CRITERION OF EXISTENCE OF A STATE OF ANTINEURITIC VITAMIN B DEFICIENCY. In selecting a suitable criterion by which to judge whether a state of antineuritic B deficiency exists it was decided to consider the appearance of the subtle anorexia, that develops in the absence of this vitamin, as the sign that the organism's requirement for this accessory factor is not being met. This criterion has several advantages. In the first place, it is usually the earliest sign to appear. Therefore, exhibition of it by experimental animals means in most cases that the state of vitamin deficiency existing in the given animal is relatively mild: the organism is

² Personal communication.

³ From the Harris Laboratories, Tuckahoe, N. Y.

not therefore in an extremely abnormal condition as is the case when the lack of vitamin is so great as to result in neuromuscular manifestations and degenerative changes. In our experience failure of this symptom to develop is of quite rare occurrence. Furthermore, in our earlier vitamin experiments of a quantitative nature, where this anorexia was selected as the physiological sign to be watched for, quite uniform results were obtained; this was in contrast to the wide variations shown by the data secured when cure of the serious neuromuscular manifestations was the criterion employed. We soon became impressed with the fact that it is much more difficult to compare with desired exactitude different pigeons or dogs with respect to severity of the state of which the convulsions, so-called polyn neuritis, etc., are the symptoms.

In applying the anorexia criterion to the pigeon our feeding technique was so planned as to insure an adequate daily intake of all known dietary essentials except energy, which was supplied by polished rice, and antineuritic vitamin B which was furnished by the yeast vitamin concentrate in carefully measured amounts. The birds were weighed daily. When a steady daily loss in body weight occurred, it was reasoned that anorexia had developed and the bird was not eating the amount of rice necessary to meet its energy requirement. The dose of vitamin concentrate given daily was then increased by a carefully measured amount and the potency of the preparation in checking the decline in weight was studied.

The pigeon data reported in this paper were obtained in 1926. Williams and Waterman (1928) and Eddy, Gurin and Keresztesy (1930) have since claimed that birds require a hitherto unrecognized third water-soluble factor. Re-examination of our diet and general feeding plan in the light of these reports indicates that, if such a factor does exist, our diet contains it. The details of our pigeon feeding technique have already been described (Block, Cowgill and Klotz, 1932).

The technique used with the dog has already been described (Cowgill, Deuel and Smith, 1925). If care is taken not to offer the animal too much food, the apparent anorexia due merely to adjustment of the dog's food intake to its energy demands (Cowgill, 1928) may be avoided, and the value of carefully measured doses of the vitamin preparation in preventing the appearance of the characteristic anorexia due to lack of vitamin B may be readily estimated.

In the case of rats and mice, failure of the growing animal to make its normal gain in body weight over two weighing periods—one week—was regarded as indicating that anorexia had set in, resulting in insufficient food intake. The dose of vitamin concentrate being administered daily was then increased by a measured amount. It is of interest to point out here that the vitamin measurements made in rats and mice pertain to growing animals whereas those on the pigeons and dogs were made on

adult animals. This point is of significance when endeavoring to interpret the entire mass of data and will be discussed later.

In performing quantitative experiments of the type described in this paper it is very important that one estimate the *minimum* amount of vitamin-containing material required by the test animals. Cases where an appreciable excess over and above the minimum necessary amount has been given are of little or no value for attaining the objective of this research.

These experiments were completed in 1926 when our supply of lot 985 of Yeast Vitamine Powder (Harris) became exhausted. At that time it became evident from the work of Smith and Hendrick (1926), Goldberger, Wheeler, Lillie and Rogers (1926) and others that the different biological properties hitherto attributed to vitamin B are really due to more than one substance. What was formerly designated vitamin B may well be called the vitamin B complex, in which occur at least two substances, the antineuritic factor and the heat-stable "antipellagra" vitamin. It becomes necessary, therefore, for workers in this field to differentiate, if possible, between these dietary essentials. This is especially true with reference to the quantitative studies reported in this paper, and is the chief reason why publication of these data has been delayed. The relation of each of these components, B and G, of the vitamin B complex to the development of the subtle anorexia used as the criterion of vitamin B deficiency in these experiments has now been studied (Cowgill, Rosenberg and Rogoff, 1931a; Burack and Cowgill, 1931). The results obtained suggest very strongly that lack of the antineuritic vitamin B is the chief if not the sole reason for development of this characteristic loss of the urge to eat; the pellagra-preventive factor G appears to play no part. This conclusion is further substantiated by the findings of Sherman and Sandels (1931). The data obtained in the quantitative studies reported in this paper may therefore be regarded as applying primarily, if not entirely, to the antineuritic component of the B complex.

EXPERIMENTS ON THE PIGEON

GEORGE R. COWGILL AND B. H. KLOTZ⁴

TECHNIQUE. Polished rice was offered *ad libitum*. Inasmuch as this material is poor in protein and salts as well as vitamins in general, a daily forced administration of an artificial food mixture contained in a "000" gelatin capsule and designed to satisfy the protein and other minima was

⁴ The complete details of these experiments are given in the thesis presented in 1926 by B. H. Klotz to the faculty of the Yale School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine.

made. The artificial mixture contained commercial meat residue⁵ as a source of good protein relatively free from antineuritic vitamin B (Osborne and Mendel, 1917; Cowgill, 1927), the Osborne-Mendel (1917) salt mixture and cod liver oil. The question as to the adequacy of this diet has already been discussed (Block, Cowgill and Klotz, 1932). In view of the pigeon's habit of scattering its food, it was decided not to attempt to measure the amount voluntarily eaten by the birds. Instead, the appetite was followed by observing at frequent intervals the body weight which quickly and distinctly declines with failure to eat.

Twenty-three pigeons of mixed breeds and weighing from about 300 to 635 grams were used in these experiments. For the first two weeks all of the birds received only polished rice, stone grit and water *ad libitum*. This was done in order to deplete the tissue store of vitamin B (Osborne and Mendel, 1923). Our experience indicates that about ten days' subsistence on a diet lacking the antineuritic vitamin B is necessary in order to exhaust the pigeon's supply of this dietary essential. Since the completion of this study, Pilcher and Sollmann (1925) have reported a similar finding. After this preliminary vitamin-depletion period the artificial mixture of meat residue, salt mixture and cod liver oil was administered in a gelatin capsule, one capsule being given each bird daily. When the administration of the supplementing artificial mixture was begun, the birds were classified in three groups according to body weights and the vitamin powder given daily to these groups in 30, 60 and 90 milligram doses respectively.

RESULTS. As indicated on the illustrative curves presented in chart 1, the doses of vitamin first used were too small and the birds declined markedly in weight. It became necessary to give a few enormous doses of vitamin—see chart 1, "large doses of yeast"—in order to save the pigeons and to allow them to regain the weight that had been lost. Larger doses of vitamin than those originally selected were then given and changed from time to time until the amount required to maintain the body weight at a practically constant level for a week or more was found. For example, the dose for bird 27, chart 1, was finally determined to be 90 milligrams per day for a body weight of 420 grams; the correct daily dosage for bird 38 was considered to be 100 milligrams for a body weight of 465 grams. In the same way the data summarized in the last two columns of table 1 were obtained. It will be noticed that following the administration of enormous doses of vitamin long periods approximating three weeks elapsed before making the slight changes in doses in the effort to find the minimum. This long wait was intentional, its object being to avoid any hang-over effect of the large doses previously administered.

⁵ From the Valentine Meat Juice Company, Richmond, Virginia.

At the close of the experiments most birds, particularly those whose body weights had been on the decline for several days, received 1 gram of the vitamin powder and all but one of them showed a definite and immediate response by an increase in body weight. For an illustration of this see the curve for bird 27 in chart 1.

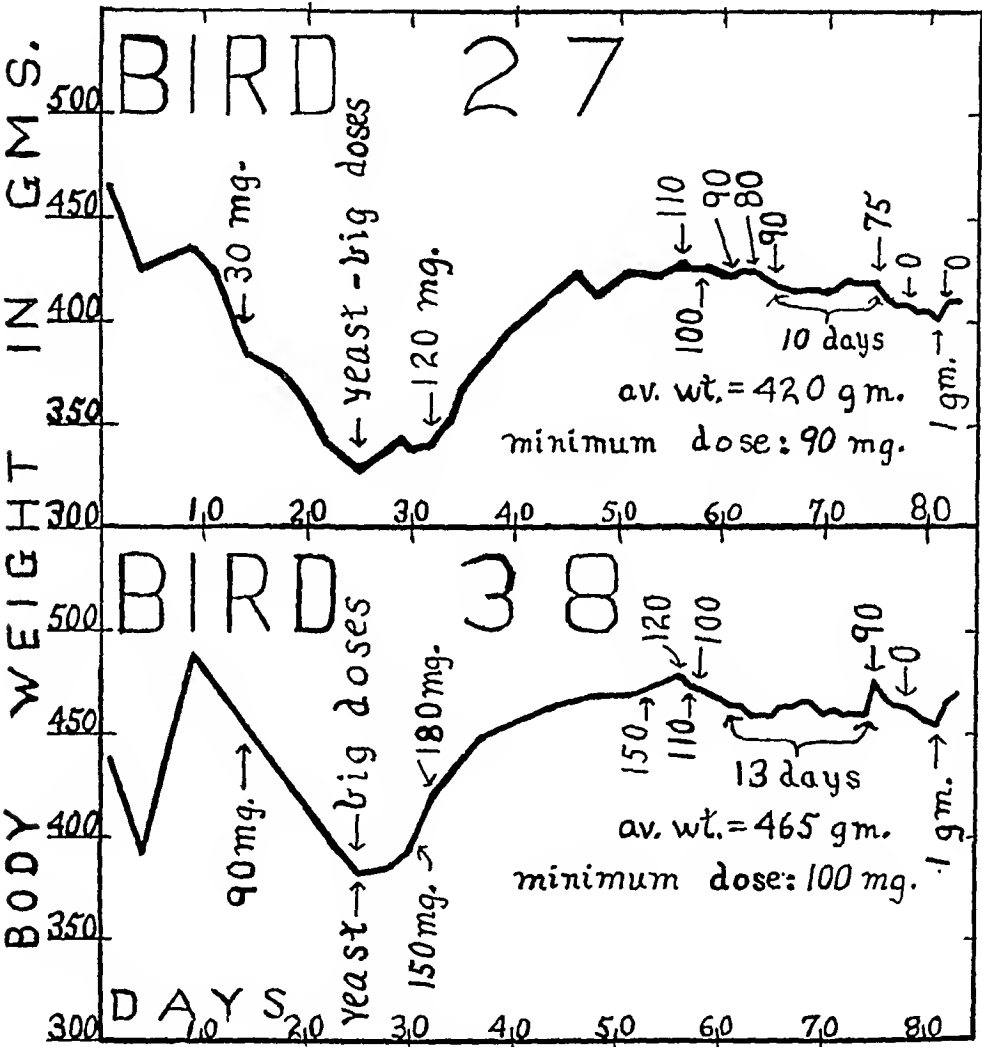


Chart 1

In table 1 are presented the data on the vitamin minimum for each bird together with the body weight and length of period over which the weight remained practically constant. In the case of pigeon 39 the vitamin minima for two quite different body weights were accurately determined. The data pertaining to both of these weights are included in this table. It will be noticed that there is a general increase of the vitamin minimum

associated with rise in body weight. Only two birds—no. 25 and no. 31—are exceptional in this respect. It is quite possible that these pigeons had either poorer utilization or greater loss of vitamin through excretory channels than the other birds.

If the data on vitamin per day—last column of table 1—are plotted as ordinates with corresponding body weights as abscissae, the resulting graph suggests that the vitamin minimum is a function of some power of

TABLE 1

BIRD NUMBER	PERIOD OF PRACTICALLY CONSTANT BODY WEIGHT	BODY WEIGHT AVERAGE	VITAMIN PER DAY AVERAGE
	<i>days</i>	<i>grams</i>	<i>mgm.</i>
40	20	300	40
22	7	310	60
37	18	350	60
25	10	390	120
41	7	410	80
23	13	415	80
30	20	420	85
27	17	420	90
39	14	420	85
42	17	420	90
44	18	440	95
33	30	440	100
39	6	450	100
38	18	465	100
32	10	465	100
31	9	485	130
34	14	500	120
26	42	510	120
48	9	520	120

TABLE 2

ALGEBRAIC EXPRESSION TESTED	VALUE OF n FIXED AT	CALCULATIONS	
		Mean K	Average deviation in per cent of mean
$\frac{VIT}{W^n} = K$	1	12.1	15.9
	2	1.60	12.6
	3	2.10^{10-1}	9.7
	4	2.78^{10-2}	6.9
	5	3.70^{10-3}	5.5
	6	4.91^{10-4}	5.8
	7	6.55^{10-5}	7.4
	8	8.76^{10-6}	10.4
	9		
	10		

the body weight. An effort was made to determine what this power might be.

Reference has already been made to the possibility that the vitamin B requirement stands in some relation to metabolism. Basal metabolism is closely proportional to body surface area (or "active protoplasmic mass" for which surface area may be taken as a good index), and body surface area is a fairly close function of the two-thirds power of the weight (Rubner, 1883; Pfaundler, 1916). This suggests that the value $\frac{2}{3}$ might be the desired power. This idea was tested in the manner described below, the

test being made more rigid by basing the calculations not only on this value but others as well. The data contained in table 1 for body weight and corresponding vitamin minimum were used to test an expression of the type

$$(1) \quad \frac{\text{Vitamin}}{\text{Weight}^n} = K$$

where n was varied from $\frac{1}{3}$ through $\frac{2}{3}$, $\frac{2}{3}$, $\frac{4}{3}$ up to and including $\frac{5}{3}$. The values for K yielded in each series of calculations based upon a given fixed value of n were compared and the degree of variation noted. Examination of the means and the average deviations from the mean of the respective series indicated what value for n of those tested gave the best agreement of values for K .

In table 2 are presented the results of such calculations. It will be noticed that the best agreement of the values of K is obtained when n is fixed at $\frac{5}{3}$. The average deviation for this series is ± 5.5 per cent of the mean. Tables 1 and 2 therefore indicate that the adult pigeon's vitamin B requirement in terms of lot 985 of Yeast Vitamin Powder (Harris) may be approximated quite accurately by the expression

$$(2) \quad \text{Pigeon: VITAMIN B}_{\text{mgm. per day}} = 0.0037 \text{ WEIGHT}_{\text{gram}}^{\frac{5}{3}}$$

Discussion of this expression will be given later when the data from all of the species can be considered.

EXPERIMENTS ON THE DOG

GEORGE R. COWGILL, ARTHUR H. SMITH AND H. J. DEUEL, JR.

In 1923 Cowgill and Deuel reported that dogs require approximately 40 milligrams of the yeast vitamine powder (Harris) per kilo body weight per day to maintain perfect appetite over periods of several months duration. These experiments were described in detail in a later paper (Cowgill, Deuel and Smith, 1925). Inasmuch as these tests required much time for their proper performance and the supply of lot 985 of the vitamin concentrate was diminishing rapidly, it was deemed advisable to pursue the problem further with other species of animals rather than to repeat and extend the work done with the dog. Furthermore, until some clues had been obtained suggesting a different line of experimentation on the dog useful in attaining the main objective of this research, further studies with this species did not seem advisable. In reporting the work accomplished it will be noticed above that the requirement for vitamin was expressed simply in terms of body weight.

Our pigeon data obtained later indicated that it might be worthwhile to review the dog data, paying particular attention to the body weight and

vitamin dosage of each animal. This was done. In table 3 are given the daily intakes of vitamin powder for six dogs ranging from 4.5 to 11 kilograms body weight. In every case the behavior of the animal at the end of a period of at least three months was such as to indicate its vitamin intake to be *very close* to the minimum required. Certainly very little excess vitamin was being ingested daily. This is a very important point worthy

TABLE 3

DOG NUMBER	BODY WEIGHT*	VITAMIN CONCENTRATE PER DAY*
	grams	mgm.
44	4,550	90
45	6,000	165
41	8,000	232
55	9,850	360
53	10,000	360
39	11,000	384

* Body weights and vitamin dosages are expressed in grams and milligrams respectively because these units are used with the other species studied.

TABLE 4

DOG NUMBER	$\frac{\text{VITAMIN}}{\text{WEIGHT}^n} = K$							
	$n = \frac{1}{3}$	$\frac{2}{3}$	1	$\frac{4}{3}$	$\frac{5}{3}$	2	$\frac{7}{3}$	$\frac{8}{3}$
		10^{-1}	10^{-2}	10^{-3}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
44	5.43	3.28	1.98	1.25	7.21	4.35	2.63	1.58
45	9.08	5.00	2.75	1.51	8.33	4.58	2.52	1.39
41	11.60	5.80	2.90	1.45	7.25	3.68	1.81	0.91
55	16.80	7.84	3.66	1.71	7.76	3.71	1.73	0.81
53	16.71	7.76	3.60	1.67	7.96	3.60	1.67	0.78
39	17.30	7.76	3.49	1.57	7.06	3.17	1.43	0.64
Mean.....	12.82	6.24	3.06	1.52	7.60	3.84	1.97	1.03
Average deviation....	4.11	1.55	0.52	0.12	0.42	0.42	0.41	0.31
Average deviation in per cent of mean...	32.1	24.8	17.0	7.90	5.53	10.9	20.8	30.1

of emphasis. Many other dogs were experimented with but the data yielded by them cannot be considered here because the animals were undoubtedly receiving a considerable excess of vitamin above the minimum required for physiological well being.

If the expression

$$(1) \quad \frac{\text{Vitamin}}{\text{Weight}^n} = K$$

be tested with the data contained in table 3 with the view to finding the value of n that gives the best agreement of the values of K by varying n through $\frac{1}{3}$, $\frac{2}{3}$, etc., as was done with the data for pigeons, the data presented in table 4 are obtained.

Examination of table 4 shows that there is the least variation in values of K when the exponent of the weight is $\frac{5}{3}$. The average deviation in per cent of the mean in this case is ± 5.53 which is almost identical with that for the pigeons, namely, ± 5.5 (see table 2). From the data presented in tables 3 and 4 it appears that the adult dog's daily vitamin B requirement in terms of lot 985 of the yeast vitamin powder (Harris) may be approximated quite accurately by the expression

$$(3) \quad \text{Dog: VITAMIN B}_{\text{mgm. powder per day}} = 0.000076 \text{ WEIGHT}_{\text{gram}}^{\frac{5}{3}}$$

Comparison of this expression with that obtained for the pigeon reveals a striking similarity. Discussion of this formula will be deferred until all of the data for the several species have been presented.

EXPERIMENTS ON THE RAT

GEORGE R. COWGILL AND ARTHUR H. SMITH

CRITIQUE. Entirely satisfactory experiments designed to determine the amounts of vitamin B just sufficient to allow physiological well being in rats of different sizes are not easy to plan. Osborne and Mendel (1922) gave different groups of young rats definite constant amounts of dried yeast as the source of vitamin B over a period of approximately a year and compared the rates of growth exhibited by the several groups of animals. Small doses limited growth to a marked extent; larger doses were associated with growth somewhat below normal and approximately the same as that usually obtained on complete rations of the type used. Sherman and Spohn (1923) utilized this scheme in developing their method of biological assay of foods for vitamin B. Although such a plan of experimentation yields valuable data and serves to demonstrate in striking fashion how the intake of vitamin B may be a limiting factor in growth, in our opinion it cannot give data that can be compared satisfactorily with those obtained from the pigeon or the dog. Consider the rats to which Osborne and Mendel gave a daily dose of 25 milligrams of dried yeast. It took over seven months for any of these animals to attain a weight approximating 180 grams. Is it permissible to regard animals that have endured such a repression of growth as "normal" or justly to be compared with others that have not been so repressed? It is doubtful if this question can be answered at all satisfactorily, in view of the present state of knowledge. Mere mention of it, however, serves to emphasize at least one point to be con-

sidered in planning the quantitative experiments useful in attaining the main objective of this research, namely, that the experimental conditions under which the vitamin B minimum is to be measured should be such as to allow the animals to be in a condition as nearly normal as possible.

A second point that must be considered in planning work of the sort discussed in this paper concerns the vitamin B demonstrated to be present in the intestinal excreta. The experiments of Osborne and Mendel referred to above were not conducted under conditions where ingestion of the feces by the test animals was prevented. When a preliminary report of our work (Cowgill and Deuel, 1923) was given showing that the rat's requirement for vitamin B per unit of body weight is approximately five times that of the dog, the communication dealt with rat experiments in which coprophagy was possible. After our experiments had been completed there appeared the paper of Steenbock, Sell and Nelson (1923) calling attention to the vitamin B present in the feces and emphasizing the importance of considering this source of supply when performing vitamin B tests on animals. Their observations were soon confirmed not only in this laboratory (Smith, Cowgill and Croll, 1925) but elsewhere (Dutcher and Francis, 1923-24). Our quantitative studies on the rat were therefore repeated with the experimental animals housed in specially constructed false-bottom cages described elsewhere (Smith, Cowgill and Croll, 1925).

PLAN OF EXPERIMENTS. Young rats of approximately 60 grams body weight were allowed to subsist on a vitamin B-free ration consisting of 18 per cent extracted casein, 51 per cent corn starch, 18 per cent lard, 9 per cent butter fat and 4 per cent of salt mixture. Each animal received daily a carefully weighed amount of the lot 985 Yeast Vitamine Powder (Harris). This material was weighed on a tared crucible cover, the handle of which had been removed in order to make the cover lie flat. The error in weighing the vitamin powder was not over 0.5 milligram. The growth of the animal was followed carefully, weighings being made twice a week. When two consecutive records indicated a definite decrease in the growth rate, the daily dose of vitamin powder was increased by a fixed measured amount. When the growth rate again showed a decline, the amount of vitamin was increased once more.

One advantage of this procedure lies in the fact that the animals are not allowed to become seriously ill, nor are they forced to endure a marked suppression of growth. This technique has one serious disadvantage. It is often quite difficult, in some cases impossible, to determine just when the growth rate begins to decrease. The break or change in the graph cannot be definitely fixed. The only way this difficulty can be circumvented seems to be to perform a number of experiments sufficiently large to allow one to disregard the doubtful cases.

It was reasoned that the decrease in the growth rate was due in the first instance to failure to eat an amount of food sufficient to permit normal growth, and the failure to eat was due to a lack of vitamin B. In other words, appearance of the anorexia characteristic of vitamin B deficiency is the criterion that the need for this vitamin is not being met. Inasmuch as the failure to maintain growth was exhibited over a relatively short period, the condition of the animal could not be regarded as differing seriously from the "normal."

It is possible to take the view that in these experiments performed in 1923, the decrease in growth rate was due to an insufficient supply of the heat-stable pellagra-preventive substance, rather than to a shortage of vitamin B. As indirect evidence against this view we may cite 1, the observations of Sherman and Sandels (1929) that the "antipellagra" factor is difficult to extract from commercial casein, the source of protein fed by us, by means of varying concentrations of ethyl alcohol, and 2, the finding (Cowgill, Rosenberg and Rogoff, 1931; Buraek and Cowgill, 1931; Sherman and Sandels, 1931) that the loss of appetite characteristic of a shortage of the vitamin B complex is to be attributed to lack of the antineuritic B factor and not the "pellagra-preventive" vitamin G. It is likely, therefore, that our extracted casein contained appreciable amounts of the heat-stable G vitamin. Inasmuch as the first indication of change in the growth rate is the sign looked for, and this is a prompt result of anorexia, it is considered more likely that the data obtained with these rats pertain to the antineuritic vitamin B.

RESULTS. The first set of experiments was performed with a group of eleven rats (nos. 42 to 53 inclusive, table 5). These animals were used only to determine the body weight to which young rats might go when given 20 milligrams of the vitamin powder per day. It was the behavior of these animals and a study of their growth curves that suggested the idea underlying the technique described above. These rats showed considerable variation with respect to the body weight levels at which the growth rate changed and at which the vitamin dosage had to be increased. Partly to increase the number of observations and partly to determine whether, on the basis of the experience now gained, the accuracy might not be increased and the variation thus diminished, another group of nine animals was started. The mean body weight for the twenty milligram dose and the average deviations in per cent of the mean for the two series of experiments proved to be almost identical. From this it was concluded that further experimentation using this technique with the view to decreasing the average variation by increasing the number of cases was not worth while.

Data were secured from a total of twenty rats given twenty milligrams of the vitamin concentrate per day until a definite change in growth rate occurred, then thirty milligrams and lastly forty-five milligrams per day.

It proved possible with all the rats to fix with reasonable accuracy the body weight at which growth began to decrease when the animals were given the twenty milligram dose; in six cases the point was fixed for the thirty milli-

TABLE 5

RAT	YEAST VITAMINE CONCENTRATE DOSAGE OF		
	20 milligrams per day	30 milligrams per day	45 milligrams per day
Body weight reached			
	grams	grams	grams
856	108	143	158
857	104	149	?
855	103	?	136
852	102	126	?
859	101	132	?
854	94	118	?
858	92	?	128
860	88	126	148
853	87	?	?
44	110		
42	108		
43	106		
45	102		
53	101		
52	100		
51	95		
48	92		
46	90		
49	86		
47	80		
Total cases...	20	6	4
Average weight.....	98	132	143
Average de- viation in per cent of mean.....	7.1	5.5	7.3

TABLE 6

ALGEBRAIC EXPRESSION TESTED	VALUE OF n FIXED AT	CALCULATIONS BASED ON ALL 30 CASES		CALCULATIONS BASED ON AVER- AGE WEIGHTS FOR EACH GROUP	
		Mean K	Average deviation in per cent of mean	Mean K	Average deviation in per cent of mean
$\frac{VIT}{W^n} = K$	1	5.2	22.4	6.3	24.5
	1.1	1.1	17.0	1.3	21.6
	$2.3^{10^{-1}}$	2.3 ^{10⁻¹}	13.6	2.5 ^{10⁻¹}	18.4
	$4.7^{10^{-2}}$	4.7 ^{10⁻²}	12.7	5.0 ^{10⁻²}	14.0
	$9.9^{10^{-3}}$	9.9 ^{10⁻³}	13.0	9.9 ^{10⁻³}	9.0
	$2.1^{10^{-3}}$	2.1 ^{10⁻³}	15.9	2.0 ^{10⁻³}	10.0
	$4.4^{10^{-4}}$	4.4 ^{10⁻⁴}	19.6	4.0 ^{10⁻⁴}	10.8
	$9.0^{10^{-5}}$	9.0 ^{10⁻⁵}	22.2	8.2 ^{10⁻⁵}	13.0

gram dose, and in four cases for the forty-five milligram daily allowance of yeast concentrate. The data are presented in table 5.

The 30 cases listed in table 5 were examined with the view to determining the best value for the exponent of the body weight in the expression

similarly tested on pigeons and dogs. The average body weights for the different vitamin doses were also used for such a test. The results of these calculations are presented in table 6.

In the calculations (table 6) based on all 30 cases, it will be noticed that the best value for n , as shown by the minimum average deviation, is $\frac{4}{3}$, and that the average deviation in per cent of the mean in this case is only very slightly lower than that obtained when n is $\frac{5}{3}$. If the average body weight values for the three vitamin dosages are used for the calculations (last two columns of table 6) $\frac{4}{3}$ is clearly indicated as the best value for n , giving an average deviation of 9 per cent of the mean value of K which is 0.0099. The following expression may therefore be formulated:

$$(4) \quad \text{Rat: VITAMIN}_{\text{mgm. per day}} = 0.0099 \text{ WEIGHT}_{\text{gram}}^{\frac{4}{3}}$$

This expression is obviously applicable to rats ranging from 80 to 160 grams body weight with an accuracy of about 9 per cent. Upon initial consideration this may appear to be too great a variation. It is pertinent, therefore, to call attention to the fact that this is only slightly greater than the variation in the basal rate of metabolism exhibited by normal individuals. For example, Harris and Benedict (1919) have shown that the average basal heat production of 104 women is 850 calories per square meter of body surface per day; the average deviation is 7 per cent of this mean. In view of these considerations it appears safe to conclude that even these rat data are of significance, particularly when they point to the same type of relationship existing between body weight and vitamin B minimum as is demonstrated to occur with the pigeon and the dog. It is our belief that formula (4) constitutes a closer approach to a true mathematical statement of the rat's vitamin B requirement than first approximation expressions of the type offered earlier, for example, by Osborne and Mendel (1922), where the requirement is stated as "milligrams of yeast per 100 grams of rat."

EXPERIMENTS ON THE MOUSE

H. H. BEARD

When Beard (1925-26a) was studying the nutritive requirements of mice in this laboratory, it was suggested that he endeavor to determine the minimum amount of our lot 985 Yeast Vitamin Powder (Harris) required to allow growth in mice, using the technique previously employed on rats and described above. He encountered considerable difficulty in his experiments and was forced to repeat many feeding trials with new groups of animals. Eight instances were finally obtained where the body weights, to which mice could go on a given vitamin dosage before suffering a serious repression of growth, were observed.

In Beard's paper were presented mathematical expressions for estimating the vitamin B requirements of mice, rats and dogs in terms of his test product. We have never regarded these formulae as other than purely tentative in character, interesting to consider and subject to revision if necessary following further research. The data for the mice, when applied to the vitamin expression tested above for the other species, indicate the value of the exponent of the weight to be $\frac{7}{3}$ instead of $\frac{5}{3}$. It is our belief that the error of the work on mice is greater than that for any of the other species and that further experiments should be performed before conclusions be drawn with respect to the mouse. The accuracy of the weighing of the animals should be increased so as to give three significant figures instead of only two. Inasmuch as our supply of lot 985 of the yeast concentrate is exhausted, such additional experiments will have to be made with other material.

The data yielded by the experiments with mice are presented in table 7.

COMPARISON OF DATA FROM DIFFERENT SPECIES

GEORGE R. COWGILL

From the foregoing review of the data obtained from the different species of animal here investigated it is evident that the same formula, except for the equating constant, can be used with which to express the need for vitamin B. This vitamin requirement is in linear relation to the $5/3$ rd power of the body weight. A comparison of the several formulae shows that the value of the equating constant — K_s , or *species constant*—is greatest for the smallest species and decreases with increase in species size. For example:

$$(5) \quad \text{VIT} = K_s \cdot W^{\frac{5}{3}}$$

where

$$(6) \quad K_{\text{rat}} = 0.0099 \text{ or about } 0.01$$

$$(7) \quad K_{\text{pigeon}} = 0.0037$$

and

$$(8) \quad K_{\text{dog}} = 0.000076$$

If it be assumed that this expression is a fundamental one and that its failure with the mouse is due to greater difficulty of experimentation with this species, one can estimate from the mouse data at hand that

$$(9) \quad K_{\text{mouse}} = 0.15 \text{ approximately}$$

A plot of the data for all species on the same paper gives an interesting graph shown in chart 2. Because the values for body weight cover so wide a range, from a 14 gram mouse to an 11,000 gram dog, the logarithms of the body weights are plotted as abscissae against the logarithms of vitamin requirements as ordinates. The slopes of the plots for the individual species seem to point to a common origin, which was finally fixed more or less arbitrarily as the point -2.75 on the ordinate or y axis. The slants for the rats, pigeons and dogs agree well with such a plot; the slope for the mice does not conform so well.

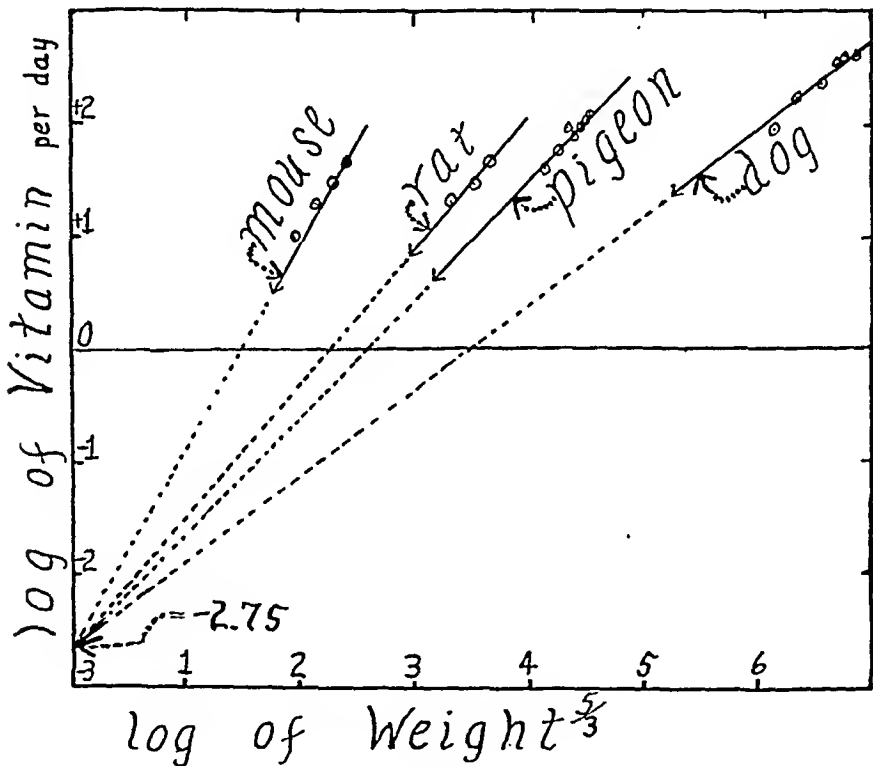


Chart 2

Inspection of chart 2 indicates the existence of a constant common to all of the species, namely, the intercept of -2.75 on the *log vitamin* axis; the values of the slopes for the various species are, of course, the ratios of this common constant, -2.75 , to the respective intercepts on the *log Weight^{5/3}* or x axis, and are peculiar to each species. From chart 2, by elimination of logarithms, one obtains an expression of the following type:

$$(10) \quad \text{VIT} = K_u \cdot [W_i^{5/3}]^{k_s}$$

where VIT signifies vitamin per day, K_u is the common or universal (?) constant, W_i is the body weight of the individual in question, and k_s

is a constant, the value of which is peculiar to the species. Expression 10, although interesting in its suggestion that there is some factor common to all of these species, does not prove immediately applicable in the solution of the main problem of this research.

A study was made of the relation between body weight and the species constants in expressions 6, 7, 8 and 9. It is obvious that if a comparison of individuals of different species is to be made on any weight basis, the individuals must be of comparable ages; just how one might determine what would be comparable ages for the individuals of the species under investigation is not clear. Suppose, however, that the *maximum normal weight* for each species be taken as the basis upon which to make a comparison. Such a comparison would be made at what might be regarded as a limiting condition. By the term *maximum normal weight* the writer means not the greatest weight ever found for that species but the average of the weights of numerous large individuals of the species. Approaching our problem from this point of view let us now inquire as to what may be taken as the *maximum normal weights* for the species here studied.

Beard (1925-26b) cited as the largest mice produced in his colony, individuals weighing 34 grams; larger normal mice have not been reported in the literature so far as the writer has been able to ascertain. Let us therefore take 34 grams as the maximum normal weight of the mouse to use in making our species comparison.

With respect to the rat, Osborne and Mendel (1926) have shown that when suitable diets are employed, individuals of this species can exhibit much more rapid growth rates and attain greater size than those observed previously. This has been confirmed by Smith and Bing (1928). Outhouse (1931) has shown that these rapidly growing animals have normal proportions of bodily parts and therefore are not pathological. In his studies of the velocity constant of growth, Brody (1928) used data furnished by Osborne and Mendel. The maximum body weight shown in Brody's⁶ chart is slightly greater than 520 grams. This figure has therefore been taken as the maximum normal weight of the rat species.

According to Dr. Stanley C. Ball,⁷ the maximum normal weight characteristic of pigeons is about three pounds or 1353 grams. In the exhibit at the Peabody Museum of Yale University the largest pigeons are designated as having this weight. The writer has therefore taken 1350 grams as the maximum normal weight for the pigeon species.

Whitney (1927) has collected a large amount of data pertaining to the weights of different breeds of dog. The average of the maximum weights for the five giant breeds, Great Danes, Newfoundlands, Mastiffs, St.

⁶ Brody, S. 1928. Missouri Agricultural Experiment Station, Research Bulletin 116. See figure 1, curve 1, page. 7.

⁷ Curator of Zoology, Peabody Museum, Yale University.

Bernards and Irish Wolfhounds was found to be 159 pounds or approximately 72 kilograms. This figure therefore was taken as the maximum weight for the dog to be used in the comparison of species.

The result of a comparison of the vitamin constants for these species (expressions 6, 7, 8 and 9) with the maximum normal weights for the respective species is shown in chart 3, where $\log K_s$ is plotted on the ordi-

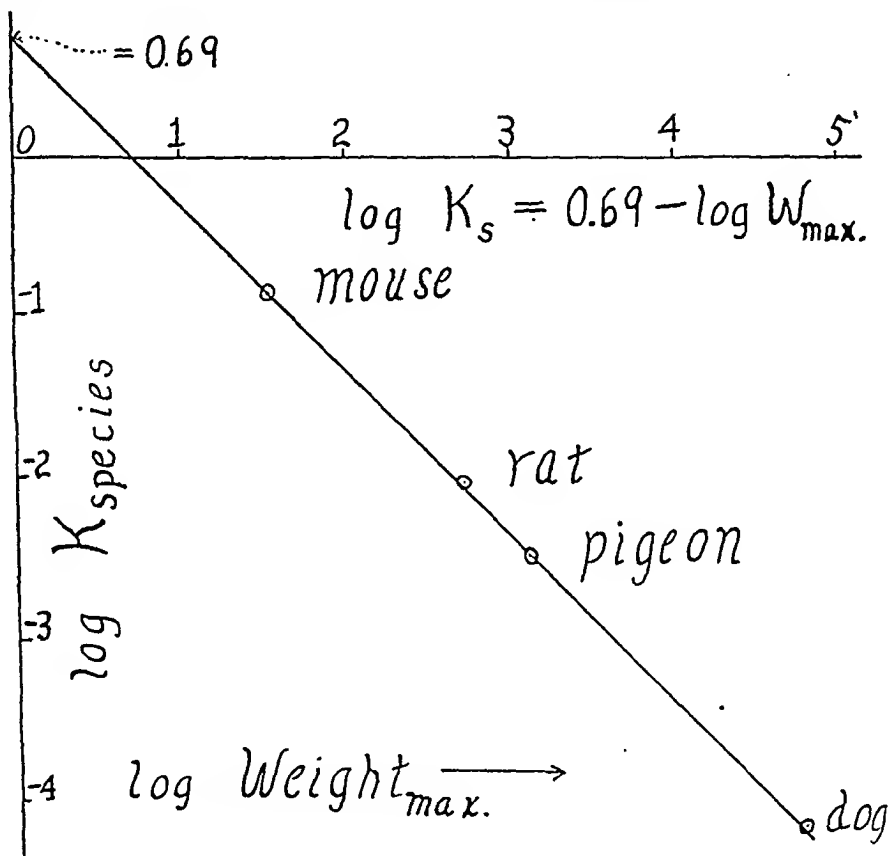


Chart 3

nate axis against $\log W_{\text{max}}$ as the abscissa. Consideration⁸ of the plot in chart 3 leads to the expression:

$$(11) \quad \text{VIT} = \frac{4.9}{W_{\text{max}}} \cdot W_i^{1.66}$$

⁸ It will be noticed in chart 3 that through the plots for the various species a line may be drawn cutting the two axes at an angle of 45° . This line intercepts the y axis at point 0.69. From this it is evident that

$$(13) \quad \log K_s = 0.69 - \log W_{\text{max}}$$

From the data for the several species it has already been shown that

$$(14) \quad \text{VIT} = K_s \cdot W_i^{1.66}$$

By writing expression 11 as given in formula 12 one obtains some simplification aiding in interpretation.

$$(12) \quad \text{VIT} = 4.9 W_i^{0.66} \cdot \left[\frac{W_i}{W_{max}} \right]$$

Expression 12, when tested with the experimental data for the several species, yields the calculated vitamin requirements given in the last column of table 8. These should be compared with the values observed experimentally presented in the adjacent column.

Considering the nature of the experimental work, particularly the difficulties encountered, the agreement of these calculated values with those observed experimentally is excellent. The greatest differences noticed are with the mice, where the error of experimentation was undoubtedly the greatest, and two pigeons and two dogs which required appreciably more vitamin than that estimated by this formula. The latter cases may be instances of individuals that have poorer utilization or storage or greater elimination of the vitamin than most cases. Minot (1929) has emphasized variability of gastroenteric function as a possible cause of variations exhibited by clinical cases in this respect.

In expression 12, the term $W_i^{0.66}$ may be regarded as indicating *metabolism*, because, as is well known, the basal metabolic rate and the body surface area are functions of the two-thirds power of the body weight. The expression $\frac{W_i}{W_{max}}$, the ratio of the individual's weight to the maximum normal or limiting weight for his species, stands in some relation to age and growth; in fact, it is one indication of the growth already attained. As the individual grows the value of this ratio approaches unity as a limit. When the value of this ratio is unity, obviously, from the remainder of the formula, the daily vitamin requirement is then dependent entirely upon the metabolism (or some unknown variable which is a function of the two-thirds power of the weight). In the case of the individual who stops growing at some weight below the maximum normal weight for his species, obviously the value of the ratio $\frac{W_i}{W_{max}}$ becomes fixed; the vitamin re-

which is equal to

$$(15) \quad \log \text{VIT} = \log K_s + 1.66 \log W_i$$

By substituting in this expression for $\log K_s$, that obtained above in expression 13, one obtains the following:

$$(16) \quad \log \text{VIT} = 0.69 - \log W_{max} + 1.66 \log W_i$$

which, by elimination of logarithms, becomes

$$(11) \quad \text{VIT} = \frac{4.9}{W_{max}} \cdot W_i^{1.66}$$

For reasons given in the text it is of advantage to write this as follows:

$$(12) \quad \text{VIT} = 4.9 \cdot W_i^{0.66} \cdot \frac{W_i}{W_{max}}$$

TABLE 7

YEAST VITAMIN CONCENTRATE PER DAY	BODY WEIGHT AT WHICH THIS AMOUNT OF VITAMIN IS THE MINIMUM
<i>mgm.</i>	<i>grams</i>
10	14
10	14
20	20
20	19
30	23
30	23
40	26
40	26

TABLE 8

$$VIT = 4.9 W_i^{0.66} \left[\frac{W_i}{W_{max}} \right]$$

SPECIES	W_{max} OF SPECIES	W_i	VIT	
			Observed	Calculated
	<i>grams</i>	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>
Mouse	34	*14	10	12
		*20	20	21
		*23	30	27
		*26	40	33
Rat	520	*97	20	19
		*129	30	31
		*153	45	41
Pigeon	1,350	300	40	49
		310	60	52
		350	60	63
		390	120	76
		410	80	82
		415	80	84
		420	85	86
		420	90	86
		420	85	86
		420	90	86
		440	95	92
		440	100	92
		450	100	96
		465	100	101
		465	100	101
		485	130	109
Dog	72,000	500	120	114
		510	120	118
		520	120	122
		4,550	90	85
		6,000	165	135
		8,000	232	218
		9,850	360	308
		10,000	360	316
		11,000	384	370

* Average.

quirement is then determined by the metabolism (or whatever the $W_i^{0.66}$ signifies) multiplied by a slightly different constant which in this case is the product of 4.9 and the constant value of the ratio $\frac{W_i}{W_{max}}$.

The significance of the ratio $\frac{W_i}{W_{max}}$ as a factor probably related to growth is enhanced when one considers the growth formula that Brody (1927) has brought forward based upon the relation of the body weight to age and to what he calls the *mature weight*. This *mature weight* is somewhat analogous to our *maximum normal weight*.⁹ The left-hand part of Brody's formula is similar to our ratio $\frac{W_i}{W_{max}}$ given in expression 12. If, in formula 12 therefore, one were to consider this ratio as indicative of age or growth, expression 12 might be regarded as meaning that the daily vitamin requirement is directly proportional to the *metabolism of the organism* (or some factor which is a close function of the two-thirds power of the body weight) *multiplied by a factor correcting for age*.

It is quite generally agreed that the characteristic symptoms of vitamin B deficiency do not appear as readily in animals subjected to *complete* starvation as in those who eat appreciable amounts of the ration deficient in this dietary essential. From this point of view the loss of appetite for the B-deficient diet may be regarded as a reaction protecting the body against development of an unfortunate syndrome. This also suggests that the *total metabolism*, or else the *metabolism of ingested food* is of greater significance here. This idea has already been tested in two ways: 1, by experiments on dogs forced to exercise on a treadmill and given an extra amount of food sufficient to allow maintenance of the original body weight while undergoing the exercise (Cowgill, Rosenberg and Rogoff, 1931); and 2, by determination of the effect of increased metabolism due to administration of thyroid tissue upon the requirement for vitamin B (Himwich, Goldfarb and Cowgill, 1931). In both of these investigations, with a rise in the total metabolism of the animals there was an increase in the vitamin B requirements. These studies indicate that under experimental conditions where, as in expression 12, WEIGHT is kept constant and CALORIES_{total}¹⁰ is varied markedly, the value for VITAMIN changes as would be expected from this formula.

It is of interest to inquire whether the data reported in this paper throw any light upon the question as to the relation of vitamin B to the metabolism of particular foodstuffs. Funk (1914), Randoin and Simonnet (1924) and others have expressed the view that this vitamin is in some way related

⁹ Brody's formula is as follows:

$$(17) \quad \frac{W}{A} = 1 - e^{-k(t-t^*)}$$

where W is the weight of the individual at time t , A is the mature weight, k is the fraction of the decline in the time-rate of growth, and t^* is the zero time from which t is determined.

¹⁰ Assuming that $W_i^{0.66}$ signifies calories total.

to the metabolism of carbohydrate. The findings of Hartwell (1928) have been cited as favoring the view that protein metabolism involves vitamin B. On the other hand, Green (1918) and Plimmer (1926) contend that vitamin B function is related to the calories metabolized irrespective of type of foodstuff. The data reported in this paper do not indicate any particular foodstuff as related in metabolism to the antineuritic vitamin. It should be pointed out that the plan of experimentation followed here is doubtless unsuited for crucial study of this question. It is of interest, however, to consider the types of mixtures that were ingested by these experimental animals. In table 9 are shown the calories contributed to the various experimental diets by protein, fat and carbohydrate 1, in the rations as prepared and offered to the animals, and 2, as metabolized, assum-

TABLE 9

DISTRIBUTION OF CALORIES AMONG PROXIMATE PRINCIPLES OF THE VARIOUS DIETS	SPECIES			
	Mouse: diet 5 C. per gram	Rat: diet 5.2 C. per gram	Pigeon: diet 4 C. per gram	Dog: diet 4.7 C. per gram
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Fraction of total calories from:				
Protein.....	25	19	12	43
Fat.....	44	28	1	31
Carbohydrate.....	31	53	87	26
Fraction of total calories assuming that 58 per cent of the protein is metabolized as carbohydrate:				
Protein.....	10	8	5	18
Fat.....	44	28	1	31
Carbohydrate.....	46	64	94	51

ing that 58 per cent of the protein is converted to carbohydrate and then metabolized.

It will be noticed in table 9 that in the case of the pigeons the amount of fat ingested was almost negligible, being only 1 per cent; the rats ate 28 per cent of their calories in the form of fat, and dogs 31 per cent. The carbohydrate calories ranged from 26 per cent in the case of the dogs to 87 per cent for the pigeons. It is an interesting fact that in spite of these wide differences in diets used, essentially the same relation of vitamin need to 5/3rd power of the body weight was found to exist in the several species. Other lines of experimentation are undoubtedly necessary in order to determine whether vitamin B function is related to the metabolism of carbohydrate or the other foodstuffs yielding energy.

It is not unlikely that expressions of types 5 and 12 will be found which state the body's need for other dietary essentials besides vitamin B. Ob-

viously the requirement of any substance that is a *constituent* of the cell—structural unit—will be directly proportional to at least the body mass, or weight; and if the substance participates in metabolism and thus disappears, the need for it will be directly proportional to the intensity of the metabolic processes in which it takes part, and therefore proportional to the metabolism of the tissue mass. If these theoretical considerations are correct, then we have in expression 5 a new approach to the problem as to what constitutes a valid basis for comparison of individuals of widely different size with respect to the metabolism of numerous ingested necessary nutrients. The writer hopes to be able to test this hypothesis in various ways.

SUMMARY

In this paper are described experiments conducted with mice, rats, pigeons and dogs, in which attempts were made to determine the amounts of the *same* vitamin B concentrate required to maintain the normal urge to eat in individuals differing widely in size and species. The quantitative data obtained for each species were examined and tested with the view to finding the best function of the body weight to use in estimating the organism's vitamin requirement. The data were found to agree well with the following expression.

$$\text{VITAMIN}_{\text{per day}} = K_s \cdot \text{WEIGHT}^{\frac{5}{8}}$$

where the value of K_s is peculiar to the species. Further study showed that the value of this species constant is inversely proportional to another variable, namely, the "maximum normal weight" of the species, and that by means of this relationship it is possible to calculate the vitamin requirements quite accurately taking into account not only the size of the individual but the species to which it belongs as well. The agreement of the calculated vitamin requirements with those observed experimentally was remarkably close in all but a few instances.

An attempt was made to interpret the mathematical expressions obtained. Considerations were advanced favoring the view that the amount of antineuritic vitamin required to maintain the normal urge to eat is proportional to the total metabolism of the individual multiplied by a factor correcting for age. If individuals within a given species are being compared, another statement appears valid, namely, that the vitamin requirement per unit of mass, i.e., *weight*, is proportional to the metabolism of that mass, that is, the *two-thirds power of the weight*.

The ultimate objective of this research was the determination of man's requirement for vitamin B. In view of the fact that the findings of this research apply to four quite different species of animals, it appears rea-

sonable to generalize from these to the human species and to assume that these results also apply to man. It is believed that the results of this research can be utilized in the determination of man's quantitative need for this vitamin. A description of attempts to determine the human requirement for this dietary essential in the light of the findings here reported is given in another paper.

BIBLIOGRAPHY

- BEARD, H. H. 1925-26a. *This Journal*, lxxv, 668.
1925-26b. *This Journal*, lxxv, 645.
- BLOCK, R. J., G. R. COWGILL AND B. H. KLOTZ. 1932. *Journ. Biol. Chem.*, xciv, 765.
- BRODY, S. 1927. *Missouri Agric. Exper. Sta., Research Bull.* 101.
1928. *Missouri Agric. Exper. Sta., Research Bull.* 116. See fig. 1, page 7.
- BURACK, E. AND G. R. COWGILL. 1931. *Proc. Soc. Exper. Biol. Med.*, xxviii, 750.
- COWGILL, G. R. 1927. *This Journal*, lxxix, 341.
1928. *This Journal*, lxxxv, 45.
- COWGILL, G. R. AND H. J. DEUEL, JR. 1923. *Proc. XIth Internat. Physiol. Congress, Edinburgh*, p. 91.
- COWGILL, G. R., H. J. DEUEL, JR. AND A. H. SMITH. 1925. *This Journal*, lxxiii, 106.
- COWGILL, G. R., A. H. SMITH AND H. H. BEARD. 1925. *Journ. Biol. Chem.*, lxiii, p. xxiii.
- COWGILL, G. R., H. A. ROSENBERG AND J. ROGOFF. 1931a. *This Journal*, xcvi, 372.
1931b. *This Journal*, xeviii, 589.
- DUTCHER, R. A. AND E. FRANCIS. 1923-24. *Proc. Soc. Exper. Biol. Med.*, xxi, 189.
- EDDY, W. H., S. GURIN AND J. KERESZTESY. 1930. *Journ. Biol. Chem.*, lxxxvii, 729.
- FUNK, C. 1914. *Zeitschr. f. physiol. Chem.*, lxxxix, 378. Also *Die Vitamine*, Wiesbaden, 1914.
- GOLDBERGER, J., G. A. WHEELER, R. D. LILLIE AND L. M. ROGERS. 1926. *U. S. Pub. Health Repts.*, xli, 297.
- GREEN, H. H. 1918. *S. Afri. Journ. Sci.*, xiv, 483.
- HARRIS, A. AND F. G. BENEDICT. 1919. A biometric study of basal metabolism in man. *Carnegie Inst. Wash., Pub.* 279.
- HARTWELL, G. 1928. *Biochem. Journ.*, xxii, 1212.
- HIMWICH, H. E., W. GOLDFARB, AND G. R. COWGILL. 1931. *Proc. Soc. Exper. Biol. Med.*, xxviii, 646. Also *This Journal*, xcix, 689.
- MINOT, G. R. 1929. *Ann. Int. Med.*, iii, 216.
- OSBORNE, T. B. AND L. B. MENDEL. 1917. *Journ. Biol. Chem.*, xxxii, 309.
1922. *Journ. Biol. Chem.*, liv, 739.
1923. *Journ. Biol. Chem.*, lviii, 363.
1925. *Journ. Biol. Chem.*, lxiii, 233.
1926. *Journ. Biol. Chem.*, lxix, 661.
- OUTHOUSE, J. 1931. Ph.D. Dissertation, Yale University.
- PFAUNDLER, M. 1916. *Zeitschr. f. Kinderh.*, xiv, 1.
- PILCHER, J. D. AND T. SOLLMANN. 1925. *Journ. Pharm. Exper. Therap.*, xxvi, 203.

- PLIMMER, R. H. A. 1926. Brit. Med. Journ., i, 239.
- RANDOIN, L. AND H. SIMONNET. 1924. Bull. Soc. Sci. Hyg. Alim., xii, 86. Also Comp. rend. Acad. Sci., clxxvi, 903. For a good review of the relation of vitamin B to carbohydrate metabolism as championed by these investigators, see their monograph *Les Donnees et Les Inconnues du Probleme Alimentaire. II. La Question des Vitamines*. Les Presses Universitaires de France, Paris, 1927.
- RUBNER, M. 1883. Zeitschr. f. Biol., xix, 536.
- SALMON, W. D. 1925. Journ. Biol. Chem., lkv, 457.
- SHERMAN, H. C. AND M. R. SANDELS. 1931. Journ. Nutrition, iii, 395.
- SHERMAN, H. C. AND A. SPOHN. 1923. Journ. Amer. Chem. Soc., xlv, 2719.
- SIMONNET, H. 1921. Bull. Soc. Sci. Hyg. Alim., ix, 69.
- SMITH, A. H. AND F. C. BING. 1928. Journ. Nutrition, i, 179.
- SMITH, A. H., G. R. COWGILL AND H. M. CROLL. 1925. Journ. Biol. Chem., lxvi, 15.
- SMITH, M. I. AND E. G. HENDRICK. 1926. U. S. Pub. Health Repts., xli, 201.
- STEENBOCK, H., M. T. SELL AND E. M. NELSON. 1923. Journ. Biol. Chem., lv, 399.
- WILLIAMS, R. R. AND R. E. WATERMAN. 1928. Journ. Biol. Chem., lxxviii, 311.
- WHITNEY, L. 1927. Chase Magazine. See table III of the appendix to the article entitled *The mating cycle of the dog*.

DIRECT INTRA-ARTERIAL BLOOD-PRESSURE READINGS IN MAN

WILLIAM DAMESHEK AND JULIUS LOMAN

From the Boston State Hospital, Boston, Mass., Division of Research, Dr. A. Myerson, Director; and the Department of Neurology, Tufts College Medical School

Received for publication December 31, 1931

In the course of an investigation into the chemistry of brain activity as measured by chemical analyses of blood from the carotid artery and internal jugular vein, we became interested in the study of the intra-arterial blood pressure by a direct method. A series of observations was carried out, first, in the comparison of simultaneous pressure readings in the various arteries (carotid, femoral, brachial) and secondly, in the comparison of direct intra-arterial brachial artery pressure with the blood pressure as obtained with the ordinary sphygmomanometer. It was felt that the ordinary sphygmomanometric readings in the presence of arteriosclerosis might be misleading and it was thought advisable to confirm or disprove that possibility.

A few instances of the estimation of direct intra-arterial pressure readings in man are recorded in the literature. These readings were in almost all instances performed at the time of a blood transfusion. Observations of this type are recorded by Faivre (1856), Albert (1883), and Volhard (1909).

Müller and Blauel (1907) were the first to compare the intra-arterial pressure with that obtained by the indirect method. Similar comparisons were made by Dehan, Dubus and Heitz (1912) and by Merke and Müller (1925). The latter authors compared the direct and indirect brachial blood pressures in two patients with fatal diseases. For the determination of the direct pressure they used maximum and minimum pressure valves. The results as obtained by the two methods corresponded closely. An excellent review of the various methods of determining blood pressure is given by McGregor (1928).

There has been some speculation in the literature regarding the question as to whether a sclerosed artery affected the blood-pressure reading by the ordinary technique. Russel (1927) considered it "a strange belief" to feel that a contracted, hypertonic muscular tube offered no more resistance to the pressure of an armlet than a normal artery. He tested a sclerosed artery obtained shortly after death and found that it offered a resistance of

100 mm. of mercury to compression. Similar experiments were performed by many authors who are cited by Janeway and Park (1910). The latter authors concluded that the error between direct and indirect readings of the blood pressure was probably not greater than 10 mm. of mercury in the presence of normal arteries and not greater than 17 mm. with arteriosclerosis. Brooks and Luckhardt (1915) on the basis of much careful experimentation with rubber tubing concluded that "the commonly accepted criteria for obtaining systolic and diastolic blood pressures do not yield correct results but give readings which are too high. . . . In soft arteries, the error is not large, but in arteries made resistant by diseases or by contraction of their muscular elements, it must be very great."

MATERIAL AND TECHNIQUE. The patients used in this study were inmates of the Boston State Hospital. The younger ones were usually cases of dementia precox, the older ones examples of senility due probably to cerebral arteriosclerosis. A few cases of general paresis were included. All studies were made in the morning, on fasting patients. The patients

TABLE 1

ARTERIOSCLEROSIS	CONSISTENCY	TORTUOSITY	RELATION TO HUMERUS
None	Soft	Absent	Not felt against bone
Slight	Thickened	Slight	Just felt against bone
Moderate	Markedly thickened	Moderate	Easily felt against bone
Marked	Extremely thickened beading	Marked	Easily felt against bone

rested for 20 to 60 minutes before blood-pressure readings were taken. Readings were made in the recumbent position, arms at sides. With the patient resting quietly, several readings (usually four) of the blood pressure were made by either a calibrated aneroid (Tycos) sphygmomanometer or a mercury (Baumanometer) instrument. The lowest reading obtained, in almost all cases the last, was the one recorded as the indirect blood-pressure reading.

The brachial artery was now carefully examined for evidence of arteriosclerosis. The standards for the presence or absence of sclerosis were adopted (table 1).

A direct reading of the intra-arterial pressure was now obtained by introducing a no. 20-gauge needle into the brachial artery at the bend of the elbow. As shown in figure 1, the needle is connected through a three-way stop-cock with 1, a 20 cc. syringe, and 2, an aneroid sphygmomanometer. When the needle enters the brachial artery, the barrel of the syringe is pushed up rapidly and systolic pulsation is seen. When this occurs, the valve on the stop-cock is turned and blood allowed to flow towards the

attached sphygmomanometer. Entrance into the latter is prevented by a glass trap (originally supplied with the Becton-Dickinson spinal fluid manometer). The trap is used either empty or filled with a solution of sodium citrate. When blood enters it, systolic and diastolic pulsations become apparent and the transmitted pressure immediately begins to be registered on the sphygmomanometer.¹ The maximum pressure is obtained within a few seconds. The larger the needle used, the more rapidly is the maximum pressure obtained. With a fine hypodermic needle (gauge 27),

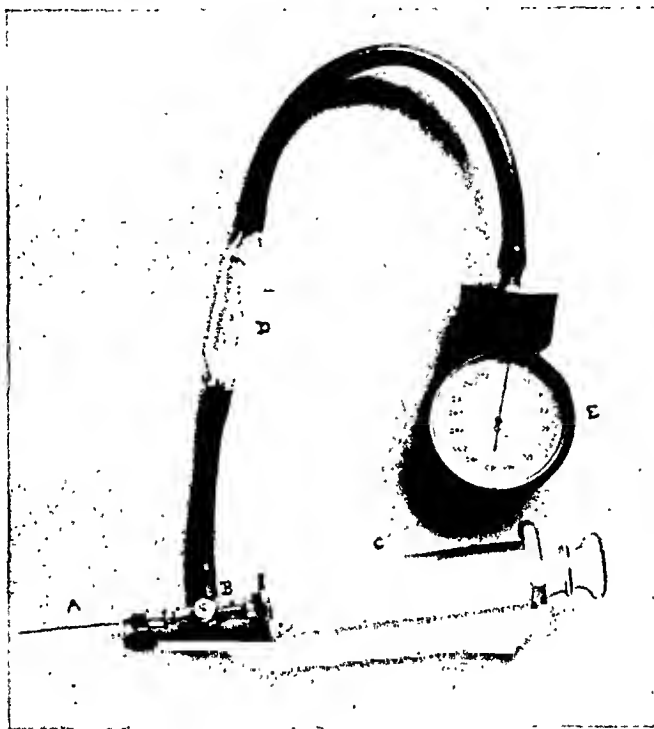


Fig. 1. Apparatus for taking direct readings of the intra-arterial blood pressure. The needle *A*, is connected through a three-way stopcock, *B*, with a syringe, *C*, and a glass trap, *D*, which prevents access of the blood to the sphygmomanometer, *E*.

the dial moves slowly to a maximum reading, which, however, is almost identical with that obtained by a large (gauge 18) needle. A certain amount of fluctuation in reading is obtained, the degree of fluctuation varying directly with the size of the needle, i.e., the finer the needle, the less the fluctuation. A wait of a few seconds is made to see whether the maximum reading has been obtained, after which the valve of the stop-cock

¹ It was found that there was too much "lag" with the mercury instrument; the aneroid manometer was found to be accurate and to check closely with readings obtained with a tight rubber dam and kymograph.

is turned to the syringe and a check made 1, as to the presence of the needle in the artery, and 2, the absence of coagulation in the needle.

Minimum readings of the intra-arterial pressure were not ordinarily made, since it was felt that they did not coincide with the diastolic pressure. It was felt that the latter pressure could not be registered because of the definite damping effect of the needle. This phenomenon and its relation to the accuracy of the technique, especially in the reading of the systolic pressure, will be discussed below.

After intra-arterial pressure readings were made, a reading of the indirect pressure was again taken. Although usually identical with the first reading of the indirect pressure, it was often lower. If lower, that reading was used for comparison with the intra-arterial reading.

Puncture of the brachial artery is not difficult. With development of the technique, puncture of the artery was in almost all instances made at the first attempt. There is practically no pain involved in a rapid puncture with a sharp needle. The emotional response on the part of the patients used was apparently extremely slight and usually absent. In only two patients of a group of one hundred fifty examined, was any excitement noted. After completion of the readings firm pressure over the artery was made. In a few patients with moderate or marked arteriosclerosis, a hematoma was formed; this could easily be controlled by pressure.

Reliability of the technique. Various objections were raised regarding the reliability of the technique used for the direct estimation of the arterial blood pressure. The most serious of these objections was that the reading as obtained by means of a needle introduced into the blood-stream was not a true measure of the systolic pressure but rather a mean pressure at an undetermined level between the systolic and diastolic pressures. Numerous experiments were therefore undertaken in an effort to determine the reliability of the technique. Two sets of experiments were in general carried out: one on human subjects and the other on an electrically operated artificial circulation machine used for teaching purposes in the laboratories of the Department of Physiology, Tufts College Medical School. At the conclusion of the experiments, it was found that the method did indeed measure mean pressure.

In experiments on the human subjects, the following factors were investigated: 1, the intrinsic "lag" of the sphygmomanometer; 2, the damping effect of air rather than fluid in the apparatus used; 3, the damping effect of the needle. It was determined 1, that the mercury manometer showed a marked lag, and that the aneroid instrument was very sensitive and without appreciable lag as checked against a tight rubber tambour; 2, that the use of air damped the fluctuations about the mean pressure but did not appreciably affect the accuracy of the readings; 3, that the finer the

needle, the greater the effect upon fluctuations about the mean pressure, with, however, only insignificant variation in the actual maximum reading obtained.

In experiments on the artificial circulation machine, it was determined 1, that use of a needle damped the fluctuation between maximum and minimum pressure; 2, that the size of the needle used had but little affect on the actual reading of the maximum, although a hypodermic needle effectively damped all fluctuation between maximum and minimum pressures; 3, that the use of water in the artificial circulation gave the same results as a solution of gum acacia isotonic with that of blood; 4, that the greater the pulse pressure the greater the difference between the reading as obtained by our technique and the actual pressure within the artificial circulation. The following data were obtained:

At pulse-pressure 20-30 mm. Hg: difference between the actual intra-arterial pressure and the pressure as determined by authors' technique was 6-8 mm. Hg.

At pulse-pressure 40 mm. Hg: difference—10-14 mm. Hg.

At pulse-pressure 60 mm. Hg: difference—20-25 mm. Hg.

At pulse-pressure 80 mm. Hg: difference—30-40 mm. Hg.

At pulse-pressure 100 mm. Hg: difference—50-60 mm. Hg.

With increasingly high pulse pressure, it was thus noted that the "maximum" pressure as determined by our technique was in reality the mean pressure.

RESULTS. 1. *Comparison of the intra-arterial pressures in different arteries.* The external carotid, brachial, and femoral arteries were compared in a small group of cases, although simultaneous readings were not taken. The three arteries were compared in eight cases. In seven instances, the brachial artery pressure was higher than either the carotid or the femoral, the difference varying between 6 and 38 mm. of mercury. In four instances, the pressure within the carotid artery was lower than that in either the femoral or brachial. In three instances, however, the femoral arterial reading was the lowest. In five cases, only two arteries were compared; in four of these the pressure in the brachial artery was higher than in the other artery.

The intra-brachial pressure was thus in almost all instances definitely higher than the intra-femoral or intra-carotid pressures. No conclusions as to whether the femoral or the carotid pressure is the lowest can be aduced from the small group of observations made. The femoral arterial pressure is probably somewhat lower than the brachial because of its greater distance from the heart and the consequent slight loss in the head of pressure.

2. *Comparison of the "direct" and "indirect" brachial artery pressures.* One hundred and forty observations were made. These were divided on

the basis of arteriosclerosis into four groups: no arteriosclerosis, slight, moderate, and marked arteriosclerosis. As noted above, the indirect pressure recorded was the lowest of several readings, either before or after intra-arterial pressure had been taken. The direct pressure represented the maximum reading on the sphygmomanometer when the above-described apparatus was used. The difference between the indirect and direct pressure readings was noted and the pulse pressure as determined from the indirect readings (by stethoscope) of the systolic and diastolic pressures was recorded.

It will be seen from table 2 that the greater the degree of arteriosclerosis, the greater was the difference between the indirect and direct blood-pressure readings. It was at first felt that this difference could be explained by the effect of the hardness of the artery on the indirect blood-pressure reading. However, when the effect of a high pulse pressure in lowering the direct blood-pressure reading was found, it was noted that the greater the arteriosclerosis, the greater the pulse pressure. This rendered ques-

TABLE 2

DEGREE OF ARTERIOSCLEROSIS	NUMBER OF CASES	AVERAGE AGE	AVERAGE SYSTOLIC BLOOD PRESSURE	AVERAGE PULSE PRESSURE	DIFFERENCE BETWEEN INDIRECT AND DIRECT BLOOD-PRESSURE READINGS
None.....	56	42.3	118.3	48.7	9.0
Slight.....	53	63.8	146.3	68.7	31.0
Moderate.....	24	73.3	168.9	84.0	45.0
Marked.....	7	70.5	196.9	110.9	62.0

tionable the surmise that indirect blood-pressure readings were inaccurate in the presence of arteriosclerosis.

DISCUSSION. This series of observations was undertaken chiefly in an effort to prove that a sclerosed artery introduced a certain definite error in the reading of the blood pressure by the ordinary methods. It was felt that a certain amount of pressure was necessary to obliterate the arterial wall before the actual intra-arterial pressure could be read. All our earlier work tended to confirm this. However, as noted above, it was found by experiments with the artificial circulation machine that our apparatus registered the mean pressure. The results on the artificial circulation machine could be closely correlated with the differences in the two techniques as observed on the human subjects if the pulse pressures were considered (table 3).

It is of interest in this connection to examine the recently published work of Livingstone, Andrews, and Adams (1931) who made simultaneous direct and indirect blood-pressure determinations in anesthetized dogs. The

indirect pressure was determined by application of a cuff placed about the hind leg and connected to a mercury manometer, the direct pressure by cannulization of the femoral or carotid artery. There was an "enormous difference" between the direct and indirect readings, the indirect being from 20 to 100 mm. higher than the direct. When the animals were gradually exsanguinated, the difference between the direct and indirect pressures gradually disappeared. The authors stated that "this tends to suggest that the determinations made on an animal with a low blood pressure are perhaps more accurate. These findings cause one to be skeptical of the height of the blood pressure in clinical cases of hypertension." The work of these authors may be criticized for the following reasons: 1, the popular type of glass cannula was employed rather than a maximum and minimum pressure valve. This type of cannula approximates the mean rather than the systolic pressure, and would therefore give a lower reading than the systolic pressure; 2, the diastolic and pulse pressures were

TABLE 3

ARTIFICIAL CIRCULATION MACHINE		MAN	
Pulse pressure	Difference—actual intra-arterial minus authors' technique, maximum reading	Pulse pressure	Difference—indirect minus direct
mm. Hg	mm. Hg	mm. Hg	mm. Hg
20-30	6-8		
40	10-14	48.7	9
60	20-25	68.7	31
80	30-40	84.0	45
100	50-60	110.9	62

not considered. The pulse pressure of dogs as obtained by several investigators working with maximum and minimum pressure valves is quite high (Allen, 1923; Kolls and Cash, 1923).

As shown above, the degree of the pulse pressure determines the difference between direct and indirect readings when a needle (and supposedly a cannula) is used. The greater the pulse pressure, the greater is the difference between the two readings. The lack of marked difference obtained by us between the two readings when the pulse pressures were low may be compared with the results obtained by Livingstone et al. when their dogs were exsanguinated. This procedure led presumably to a markedly diminished pulse pressure, and thus to an approximation of the direct and indirect readings.

The final result of our observations was to demonstrate that arteriosclerosis undoubtedly modified our intra-arterial readings, not because of the hardness of the vessel-wall, as was at first supposed, but almost certainly as a result of the increased pulse pressure which is found so commonly

in arteriosclerosis. In the face of a high pulse pressure the reading obtained was that of a modified or mean intra-arterial pressure, the modification being due to the damping effect of the needle.

Thus, our attempts to measure directly the systolic blood pressure by an intra-arterial method and to compare it with the ordinary indirect method were found in the main to be unsatisfactory. It appears extremely unlikely, therefore, that any method, aside from one utilizing the introduction of arterial cannulae equipped with maximum and minimum valves will be accurate in the direct measurement of the intra-arterial pressure. The indirect method of sphygmomanometry, as used with either the aneroid or mercury types of apparatus, appears to be an exceedingly accurate one for determining both the systolic and diastolic pressures. The possibility still remains, to be sure, that an extremely hard artery introduces a slight error in the estimation of the intra-arterial pressure by the ordinary method. Some of our results apparently tend to show this, since the difference between indirect and direct readings was greater than it should have been with the pulse pressure which was present. However, the error introduced cannot at most be more than 10 to 20 mm. of mercury, which is not sufficiently great for practical purposes.

SUMMARY AND CONCLUSIONS

1. The intra-arterial (brachial) pressure in about one hundred and fifty individuals was determined by a direct method which was compared with the usual indirect methods.

2. The method used was subjected to a large series of experiments to determine its reliability. It was finally determined that the greater the pulse pressure the less accurate was the technique used—in other words, a mean rather than a systolic pressure was determined.

3. The pressure within the common carotid, femoral, and brachial arteries was compared in eight cases. The brachial artery pressure was slightly higher than that of either the carotid or femoral arteries.

4. The apparent discrepancy between indirect and direct blood-pressure readings in arteriosclerosis was concluded to be due to the high pulse pressure which is present in that condition.

5. The indirect method of sphygmomanometry appears to be accurate even in the presence of marked arteriosclerosis.

It is a pleasure to record our thanks to Dr. A. Myerson for his numerous critical suggestions and to Dr. James V. May for his many courtesies.

BIBLIOGRAPHY

- ALBERT. 1883. *Med. Jahrb.* (cited by R. TIGERSTEDT and MÜLLER and BLAUDEL).
 ALLEN, F. M. 1923. *Journ. Met. Res.*, iv, 431.
 BROOKS, C. AND A. B. LUCKHARDT. 1916. *This Journal*, xl, 49.

- DEHAN, M., A. DUBUS AND A. HEITZ. 1912. *Mem. de la Soc. de Biol.*, lxxii, 789.
- FAIVRE. 1856. *Med. de Paris*. (Cited by R. TIGERSTEDT and MÜLLER and BLAUDEL.)
- JANEWAY, T. C. AND E. A. PARK. 1910. *Arch. Int. Med.*, vi, 586.
- KOLLS, A. C. AND J. R. CASH. 1923. *Johns Hopkins Hosp. Bull.*, xxxiv, 49.
- LIVINGSTONE, H. M., E. ANDREWS AND W. E. ADAMS. 1931. *This Journal*, xcvi, 588.
- MCGREGOR, L. 1928. *Arch. Path.*, v, 630.
- MERKE, F. AND A. MÜLLER. 1925. *Zeitschr. f. d. gesamt. exp. Med.*, xlvi, 322.
- MÜLLER, O. AND K. BLAUDEL. 1907. *Arch. f. Klin. Med.*, xci, 517.
- RUSSEL, W. 1927. *Brit. Med. Journ.*, i, 995.
- TIGERSTEDT, R. 1922. *Die Physiologie des Kreislaufes*. Vol. iii, p. 155. Berlin and Leipzig.
- VOLHARD. 1909. *Verhandl. des Kong. f. Inn. Med.*, xxvi, 200.

THE MODE OF ACTION OF ADRENIN AND THE QUANTITATION OF ADRENIN BY BIOLOGICAL METHODS

ARTURO ROSENBLUETH¹

From the Laboratories of Physiology in the Harvard Medical School

Received for publication February 17, 1932

The mode of sympathomimetic action of adrenin has not been investigated directly. There has been some discussion as to whether it acts through a physical process or through a chemical reaction but this discussion has been mainly based on theoretical arguments.

Barger and Dale (1910), after an extensive discussion of the action of adrenin and other sympathomimetic amines, concluded that "the least unsatisfactory view is that which regards the existence of stimulant activity as dependent on the possession of some chemical property, the distribution, and, in the main, the intensity of activity, as due to a physical property." Bayliss (1915) adopted a similar view. Langley (1921), on the other hand, advocated exclusively a chemical theory.

We studied the responses of the nictitating membrane (n.m.), the blood pressure (b.p.), the rate of the denervated heart (h.r.) and the inhibition of the non-pregnant uterus of the cat to varying doses of adrenalin. The mathematical treatment of the results is similar in general to that used by Hill (1910) to analyze the mode of action of nicotine and curare on the striated muscle of the frog.

METHOD. Dial (Ciba) was used to anesthetize the animals (cats).

The n.m. and the heart were denervated, either previously or acutely, the uterus always acutely. The isotonic contractions of the n.m. were recorded as described by Rosenblueth and Cannon (1932). The isometric records of the n.m. were taken by means of a spring lever; the n.m. was attached between the fixed end and the writing point so that the amplification was ten-fold. The writing point descended 9 mm. when a weight of 10 grams was applied at the point where the thread to the membrane was attached. The deformation of the spring followed a linear ratio to the weight or tension up to 25 grams. The h.r. was recorded by a mercury manometer connected with the carotid. The same method was used for the b.p., the preparation being commonly in this case that devised by Elliott (1912). The movements of the uterus were recorded by the method described by Rosenblueth (1931).

¹ Fellow of the John Simon Guggenheim Memorial Foundation.

Cocaine (7 to 10 mgm. per kilo intravenously) was sometimes used to sensitize the n.m., the vascular tissues or the uterus.

The adrenalin was injected through a glass cannula in the femoral vein. The doses injected were always diluted to make a volume of 1 cc. This was injected in 5 seconds. The injections at constant rates during longer periods of time were obtained from a burette, placed at different heights and emptying itself through a rubber tube and through varying capillary resistances into the cannula in the vein.

The superposition of the records was effected by running the kymograph back by hand.

Mathematical consideration of the curves of contraction and relaxation of the nictitating membrane and of the rise and fall of the blood pressure. The general shape of the curves of contraction of the n.m. and of the rise of the b.p. follows the equation,

$$y = k (1 - e^{-k't}),$$

where y is the height of contraction, t the time measured from the beginning of the contraction, and k and k' , constants.

The method used for verifying this equation was the following. The ordinates measured down from the asymptote (i.e., the full height of the contraction, when $y = k$) were taken at equal intervals of time; they should be in a geometrical progression.

The ratios of successive ordinates of a contraction of the n.m. on a single injection of adrenalin (i.e., in the following case, 0.008 mgm. in 1 cc. in 5 seconds) are the following:

I. One-second intervals:

1.36; 1.33; 1.31; 1.31; 1.29; 1.27; 1.31; 1.33; 1.36; 1.37; 1.37; 1.39.

II. Two-second intervals:

1.75; 1.75; 1.71; 1.69; 1.65; 1.68; 1.63; 1.73; 1.87; 1.83; 1.82; 1.87.

III. Three-second intervals:

2.29; 2.28; 2.21; 2.15; 2.13; 2.23; 2.37; 2.43; 2.50; 2.50; 2.45; 2.50.

The results are quite satisfactory. There appears, however, a certain regularity in the differences, inasmuch as the ratios first decrease and then increase, showing that the middle part of the curve is higher than the perfect exponential would require. This can be accounted for by considering that the concentration of the adrenin in the blood in which it is diluted will not be homogeneous: the first blood reaching the contracting cells will contain less adrenalin than that immediately following. Toward the end the process of contraction blends insensibly into the beginning of the relaxation, which makes it difficult to determine the correct asymptote.

It is therefore preferable to analyze the curves of longer (3 to 8 minutes) injections at a constant rate. Below are given some ratios of successive ordinates from the asymptote at different intervals of time for typical ascending curves of the n.m. and b.p. under these circumstances.

A. Ratios of successive ordinates in the contraction of the n.m.

I. Five-second intervals:

1.23; 1.24; 1.19; 1.17; 1.13; 1.14; 1.17; 1.14; 1.19; 1.14;
1.17; 1.20; 1.17; 1.19; 1.20.

II. Ten-second intervals:

1.39; 1.31; 1.29; 1.33; 1.33; 1.27; 1.31; 1.36; 1.33; 1.40;
1.39; 1.38.

III. Fifteen-second intervals: 1.56; 1.50; 1.50; 1.53; 1.48; 1.50;
1.50; 1.58; 1.60; 1.62; 1.66; 1.66.

IV. Twenty-second intervals:

1.87; 1.78; 1.85; 1.71; 1.69; 1.75; 1.71; 1.75; 1.90; 1.86.

B. Ratios of successive ordinates in the curves of rising b.p.

I. Five-second intervals:

1.13; 1.14; 1.14; 1.14; 1.12; 1.10; 1.11; 1.12; 1.14; 1.14;
1.16; 1.19; 1.17; 1.15; 1.20; 1.15; 1.20; 1.20.

II. Ten-second intervals:

1.29; 1.31; 1.29; 1.27; 1.23; 1.22; 1.25; 1.28; 1.29; 1.31;
1.36; 1.38; 1.35; 1.38; 1.38; 1.38.

III. Fifteen-second intervals:

1.48; 1.48; 1.45; 1.40; 1.37; 1.38; 1.43; 1.46; 1.50; 1.55;
1.60; 1.60; 1.62; 1.59.

IV. Twenty-second intervals:

1.67; 1.66; 1.60; 1.55; 1.54; 1.57; 1.62; 1.67; 1.78; 1.82;
1.85; 1.87.

Several curves were analyzed in this manner and they all gave comparable results.

The equation of the curves of relaxation is

$$y = ke^{-\lambda t}$$

Here the ordinates are measured from the resting level of the preparation. The causes of error pointed out before do not occur; all curves are therefore appropriate for this study. Below are given two typical examples selected at random.

A. Ratios of successive ordinates in the relaxation of the n.m.

I. Five-second intervals:

1.15; 1.15; 1.15; 1.11; 1.10; 1.10; 1.08; 1.09; 1.10; 1.11;
1.06; 1.09; 1.16; 1.10; 1.09; 1.12.

II. Ten-second intervals:

1.36; 1.37; 1.33; 1.21; 1.20; 1.20; 1.18; 1.20; 1.22; 1.21;
1.34; 1.26; 1.19.

III. Fifteen-second intervals:

1.46; 1.32; 1.46; 1.30; 1.30; 1.33; 1.30; 1.32; 1.29; 1.53;
1.33.

IV. Twenty-second intervals:

1.68; 1.42; 1.42; 1.41; 1.43; 1.44; 1.41; 1.44; 1.43; 1.43;
1.45; 1.51.

B. Ratios of successive ordinates in the fall of b.p.

I. Five-second intervals:

1.04; 1.06; 1.09; 1.08; 1.11; 1.20; 1.12; 1.13; 1.15; 1.18;
1.17; 1.12; 1.13; 1.15; 1.16; 1.21; 1.17; 1.15; 1.15.

II. Ten-second intervals:

1.19; 1.20; 1.34; 1.35; 1.26; 1.30; 1.35; 1.38; 1.31; 1.26;
1.30; 1.35; 1.41; 1.34; 1.33.

III. Fifteen-second intervals:

1.31; 1.50; 1.52; 1.45; 1.52; 1.58; 1.54; 1.48; 1.45; 1.52;
1.64; 1.63; 1.55.

IV. Twenty-second intervals:

1.59; 1.62; 1.69; 1.75; 1.70; 1.77; 1.76; 1.74; 1.70; 1.71;
1.85; 1.91.

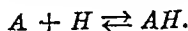
As Hill (*loc. cit.*) suggests, these two equations can be accounted for on either a physical or a chemical hypothesis. On the first assumption (*i.e.*, a physical process, diffusion, for instance) the height of contraction y would be proportional to $(A - M)$, where A is the amount of adrenin that has diffused into the smooth muscle cell, and M is the threshold amount; by A_0 is meant the concentration of adrenin in the blood (proportional to the dose injected). The equation for the contraction is then

$$y = kA_0 (1 - e^{-\lambda t}) - kM.$$

For the relaxation we would have

$$y = k' (A_0 e^{-\lambda t} - M).$$

On the chemical hypothesis adrenin combines with some constituent H of the muscle according to the reversible reaction



The height of contraction y would then be proportional to $(AH) - M$. The equation for contraction is then

$$y = \mu \left(\frac{k(A)X}{k' + k(A)} - M \right) (1 - e^{-(k' + k(A))t})$$

where $X = (H) + (AH)$ is another constant, the number of molecules

of the substance H present. k and k' are the velocity constants of the actions \rightarrow and \leftarrow respectively. For the relaxation we have

$$y = \mu [(AH)_0 e^{-k't} - M]$$

where $(AH)_0$ is the initial amount of AH present.

In the subsequent development, to simplify the formulation, the minimal amount active M will be generally disregarded. The apparent threshold for the responses is really discussable, since it can be accounted for by admitting that it represents the amount destroyed by the blood or capillary walls before it reaches the structures concerned in the response. This amount is exceedingly small when compared with the range of effective doses. Finally this suppression affects only quite incidentally the mathematic considerations.

We have then, on the physical assumption,

$$y = kA (1 - e^{-\lambda t}) \dots \dots \dots (1)$$

for the contraction, and

$$y = k'A e^{-\lambda t} \dots \dots \dots (2)$$

for the relaxation. And on the chemical hypothesis, we have

$$y = \mu \frac{(A)}{k' + k(A)} (1 - e^{-(k' + k(A))t}) \dots \dots \dots (3)$$

for the contraction and

$$y = (AH)_0 e^{-k't} \dots \dots \dots (4)$$

for the relaxation.

Since both the physical and the chemical hypotheses satisfy the shape of the curves of contraction and relaxation it is necessary to turn to other tests to decide which is correct.

The maximum of the response to different doses of adrenalin. The maximal height of contraction of the n.m. or of the increase of b.p. after a given dose of adrenalin can be very approximately obtained from equations (1) and (3) by making $t = \infty$. The error is negligible, since, from the shape of the curves, it is clear that the exponential curves of the contraction are already on their asymptotic branch before relaxation starts. This error is smaller the larger the dose of adrenalin used, and might affect only the responses to the lowest "single doses." In the constant-rate-longer injections it does not exist.

This gives (a), $y = kA$, for the physical hypothesis and (b), $y = \frac{A}{k' + kA}$,

for the chemical hypothesis. That is, according to (a) there should be a linear relation between the height of the response and the amount of adrenalin injected. On the other hand, (b) is the equation of a rectangular hyperbola whose asymptotes are parallel to the axes.

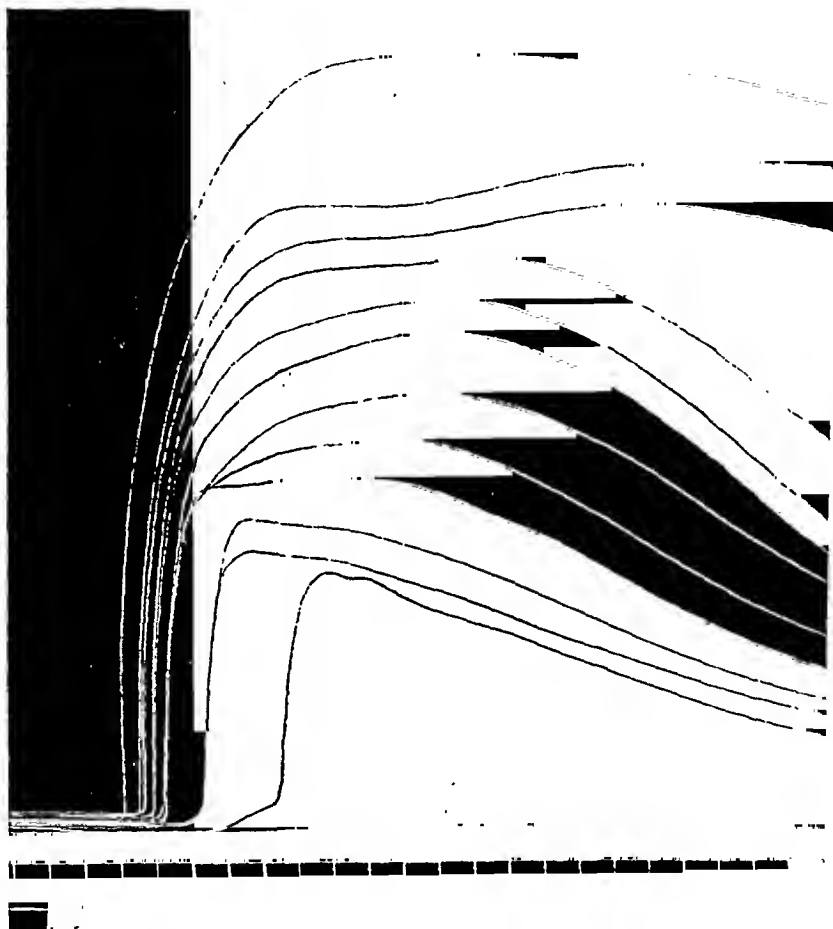


Fig. 1. Isotonic contractions of the acutely denervated nictitating membrane with the following doses of adrenalin: 0.3; 0.5; 0.7; 1; 1.6; 2.4; 3.2; 4; 6.4; 9.6; 16; 200 (unit 0.0025 mgm.). Dial, 0.7 cc. per k. intraperitoneally. Cocaine, 7 mgm. per k. intravenously. Adrenals ligated. Magnification, 14.

A and B in figure 4 show the curves obtained from the experiments illustrated in figures 1 and 2.

A simple way to test whether these curves are rectangular hyperbolas is to refer them to their asymptotes and then to test whether the product of the new y' and A' is constant. This will also show the significance of the constants k' and k in the formula.

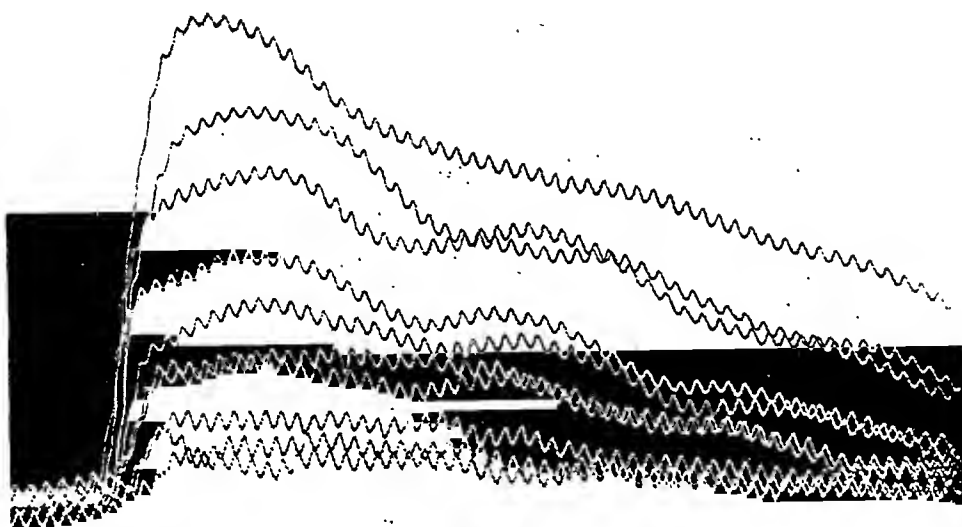


Fig. 2. Elliott preparation. Rises of blood pressure with the following doses of adrenalin: 0.3; 0.6; 0.9; 1.6; 2.4; 3.2; 4.8; 8; 12.8; 19.2 (unit 0.0025 mgm.).



Fig. 3. Isometric contractions of the nictitating membrane with the following doses of adrenalin: 0.1; 0.3; 0.6; 1; 2; 3.5; 6; 12 (unit 0.008 mgm.). Nictitating membrane denervated 10 days previously. Dial, 0.7 cc. per k. intraperitoneally. Cocaine, 7 mgm. per k. intravenously. Adrenals ligated.

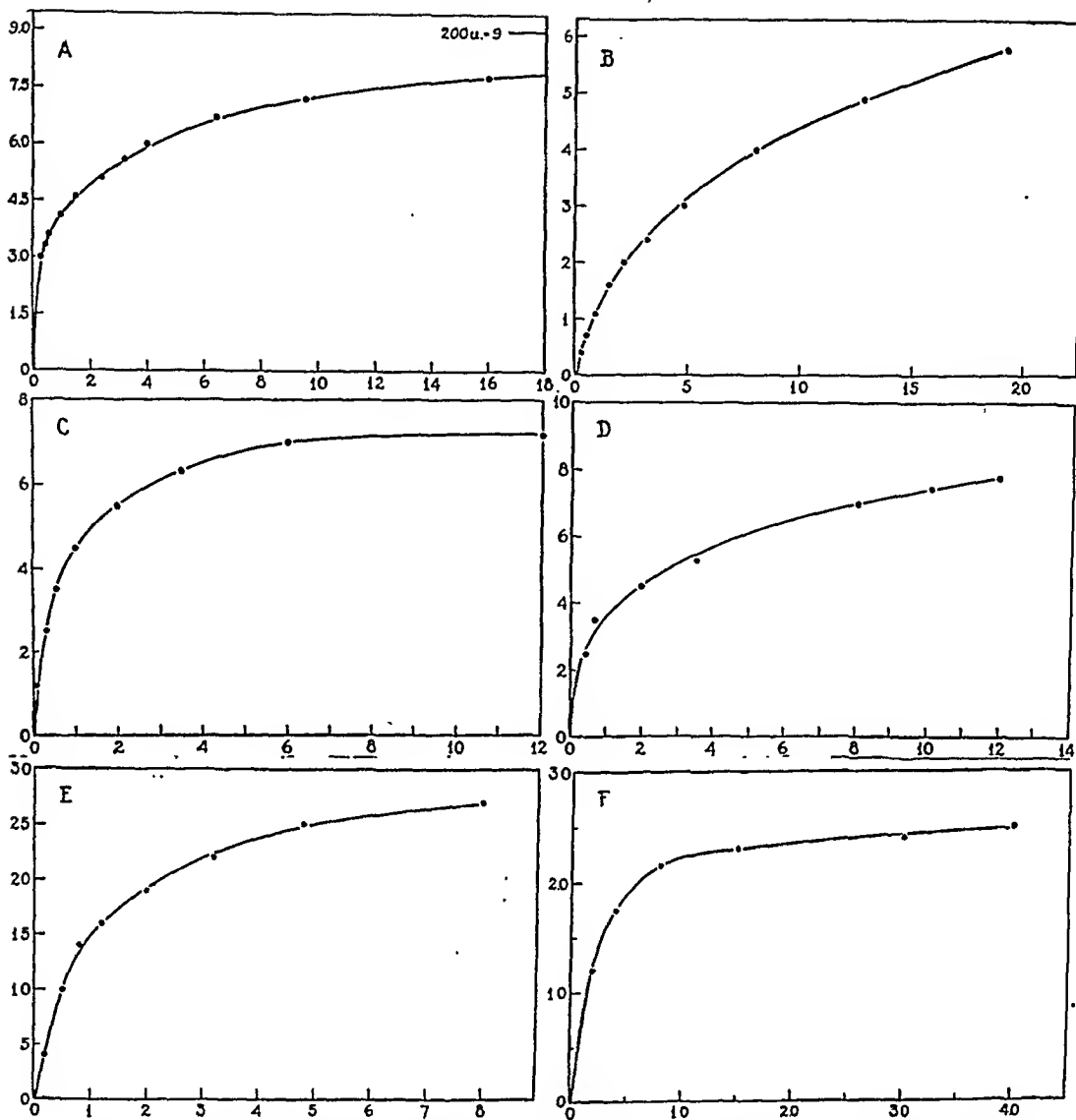


Fig. 4 A. Curve constructed from the data illustrated in figure 1. Abcissae: doses of adrenalin. Ordinates: maximal heights of the contractions of the nictitating membrane.

B. Curve constructed from the data illustrated in figure 2. Abcissae: doses of adrenalin. Ordinates: maximal rise of blood-pressure records.

C. Curve constructed from the data illustrated in figure 3. Abcissae: doses of adrenalin. Ordinates: maximal height of the contractions of the nictitating membrane.

D. Right superior cervical sympathetic ganglion removed 11 days previously. Dial, 0.7 cc. per k. intraperitoneally. Injections of different doses of adrenalin at a constant rate during variable periods (70 to 280 seconds). Abcissae: doses of adrenalin per 5 seconds (unit 0.000125 mgm.). Ordinates: average height in centimeters of the plateaus reached in the records. Isotonic contraction (magnification, 14; tension on muscle, 3 gm.).

E. Brain pithed, heart denervated acutely, adrenals ligated. Blood pressure between 100 and 130 mm. Hg, resting level. Ordinates: maximal increase of heart rate in 15 seconds. Abcissae: doses of adrenalin (unit 0.0025 mgm.). Basal rate of the heart 35 beats in 15 seconds.

F. Non-pregnant female. Dial, 0.7 cc. per k. intraperitoneally, cocaine, 7 mgm. per k. intravenously. Abcissae: doses of adrenalin (unit 0.002 mgm.). Ordinates: centimeters of relaxation of uterus in record (magnification, 15, tension on muscle, 5 gm.).

The asymptotes of the hyperbola represented by $y = \frac{A}{k' + kA}$ are $\frac{1}{k}$ (asymptote parallel to the A axis, that is, the maximal possible response of preparation) and $-\frac{k'}{k}$ (asymptote parallel to the y axis).

The significance of the constant k' appears clearly if we investigate the slope of the curve at its beginning. We have

$$\frac{dy}{dA} = \frac{k'}{(k' + kA)^2}$$

which becomes

$$\frac{dy}{dA}_{A=0} = \frac{1}{k'} = \tan \alpha$$

if we make $A = 0$ and call α the angle formed by the A axis and the tangent to the curve at its start. We see, therefore, that the slope of the curve, that is, the responsiveness of the preparation, varies inversely as k' .

Returning to equation (b) we may write

$$-yk' - ykA + A = 0$$

i.e.,

$$-\frac{k'y}{k} - yA + \frac{A}{k} + \frac{k'}{k^2} = \frac{k'}{k^2}$$

i.e.,

$$\left(\frac{1}{k} - y\right)\left(\frac{k'}{k} + A\right) = \frac{k'}{k^2}$$

This is the equation of the hyperbola referred to its asymptotes. By making $\frac{1}{k} = m$, $\frac{k'}{k} = n$ and $\frac{k'}{k^2} = c$, we have

$$(m - y)(n + A) = c$$

To test the curves it is sufficient, therefore, to find the values of m and n and to examine the constancy of c for all the values of y and A .

This procedure applied to the experiment illustrated by figures 1 and 4A gives the following results. The method of least squares yields $m = 9.13$ and $n = 42.11$. The unit of adrenin was taken as 0.0025 mgm. The maximal height of the responses was measured in centimeters from the record.

A	ν	$(m - \nu) (n + A)$
3	3.0	276.5
5	3.3	274.7
7	3.6	271.6
10	4.1	268.2
16	4.6	263.3
24	5.1	266.5
32	5.6	261.7
40	6.0	257.1
64	6.7	258.0
96	7.2	266.7
160	7.8	269.0
2,000	9.0	267.5
Average.....		266.7

Maximum deviation +9.8 and -9.6

The same procedure applied to the experiment illustrated in figures 2 and 4B gives the following results.

$m = 8.36$; $n = 94.7$. The same unit of adrenin is taken as before (0.0025 mgm.) and the rises of b.p. are measured in centimeters of mercury (i.e., the rises recorded), really one-half of the actual rise.

A	ν	$(m - \nu) (n + A)$
3	0.4	778
6	0.7	771
9	1.1	753
16	1.6	748
24	2.0	755
32	2.4	755
48	3.0	765
80	4.0	762
128	4.9	770
192	5.8	734
Average.....		759

Maximum deviation +19 and -25

Records of the isometric contraction of the n.m. were found to obey the same law (see figs. 3 and 4C).

Adrenalin injected at an even rate during longer periods of time (2 to 8 minutes) produces "plateaus" of contraction of the n.m. as well as of height of the b.p., varying in level with the amount of adrenalin injected per unit of time. The elevation of these plateaus above the resting level, plotted against the amounts of adrenalin per unit of time, yields a curve in all points similar to those described above (see fig. 4D).

The same statement can be made regarding the increases of rate of the denervated heart (see fig. 4E) and of the inhibition of the non-pregnant uterus (see fig. 4F). Several experiments were performed in each case and the results were found consistently.

The temperature coefficient of the responses of the nictitating membrane to adrenin. The value of μ in Arrhenius' formula

$$\frac{k^2}{k'} = e^{\frac{\mu}{2} \frac{T_2 - T_1}{T_2 T_1}}$$

was determined as follows. A cat under dial was moderately heated by means of a heating pad till its rectal temperature rose to 41°. Now, after removal of the heating pad, the animal was allowed to cool off while exposed to moderate cold. Under these circumstances the temperature fell within a relatively short time (1 to 2 hours) to about 35° or lower. The same dose of adrenalin was injected whenever the temperature had fallen 1°, and the contraction of the n.m. was recorded. The ratio of the velocities at two given temperatures was obtained by comparing the times necessary for the muscle to effect a given proportion (0.5 to 0.8) of its maximal contraction.

The average value of μ found was 30,000, too high for the action of adrenalin to be purely physical.

The action of cocaine. Since cocaine was used in several cases as a sensitizer, it was important to find out whether this drug modified fundamentally the results obtained by adrenin alone. It was found that cocaine does not change the formulas for the contraction and relaxation nor the curves of the maximal heights of the responses, except by changing the constants. The apparent threshold is frequently lowered (i.e., small doses, inactive before cocaine, are afterwards effective). Contraction and relaxation become longer. The maximum of the response to given doses of adrenin becomes higher and the maximal contraction possible (the horizontal asymptote of the hyperbola) also becomes higher.

The quantitation of adrenin by biological methods. If the hyperbolic shape of the plots of the responses against the doses of adrenin holds true for all organs it is to be expected that any structure sensitive to adrenin will be adequate for the quantitation of an unknown solution by comparing its effects with those of solutions of known concentration. The great variety of methods which have been proposed confirms this statement: e.g., blood pressure, excised arterial rings or strips, perfusion of the vessels of the frog's leg or of the rabbit's ear, the excised frog's iris, the denervated iris of the cat, excised rabbit's intestine or uterus.

The best indicator will evidently be the most sensitive (i.e., the most responsive to small doses), the most accurate (i.e., that with which the

measurements will be most exact and with which a given dose of adrenin will produce most nearly identical quantitative effects), the most specific (i.e., that with which foreign substances mixed with the adrenin in the solution to be tested will have the least interfering action), the simplest in its preparation, and in some cases the most lasting.

Trendelenburg (1924), who reviewed all the methods proposed up to the time when he wrote, came to the conclusion that Elliott's blood-pressure method for the quantitation of adrenin is unquestionably better than other methods, pharmacological and colorimetric, when the doses of adrenin to be determined are not too small, for it is rapid and very precise.

Rosenblueth and Cannon (1932) were struck by the precision with which the n.m. responded to a given dose of adrenin. In the experiments here reported there was ample opportunity to study the n.m. as a quantitative indicator of adrenin and to compare its responses with those of the blood pressure.

The sensitiveness of the previously denervated n.m. to adrenin is about the same as that of Elliott's preparation. The n.m. presents, however, one advantage, the very small doses of adrenin which in the b.p. method yield responses difficult to match and to estimate, produce in the n.m. quite definite and regular contractions (compare figs. 1 and 2). Furthermore, the accuracy of the n.m. method is superior to that of the b.p., for the measurements are more precise, since the n.m. records a smooth line free from the oscillations of a blood-pressure tracing.

It is not possible to establish a comparison between the relative specificity of the n.m. and the b.p. responses to adrenin. The n.m. responds to several other substances (Rosenblueth, 1932), but so does the b.p. It can only be stated that neither method is adequate for qualitative determination of adrenin.

The preparation in the n.m. method is simple. For stronger solutions of adrenin it is enough to inject intravenously 7 mgm. cocaine hydrochloride per kilo into a cat under an adequate anesthesia (dial, for instance), and to attach the membrane to the writing lever. For higher dilutions it is necessary to denervate the membrane previously (at least 5 days) by removal of the superior cervical sympathetic ganglion. In these cases, especially if cocaine is added to increase the sensitiveness (already very high), the preparation may be relatively unstable because of a certain amount of spontaneous contraction. It is then advisable to ligate the adrenals and sometimes to deepen the anesthesia. It is rare to have to discard a preparation as useless. In all these aspects the n.m. is a better indicator of adrenin than is the b.p.

The denervated heart is an excellent indicator of adrenin; the most sensitive of all according to the evidence presented up to the present, but it is a more complicated preparation than the others. In this case the

choice of an anesthetic is difficult, for almost all of them have a limiting or "fixing" action on the heart (dial, for instance) which results in a small range of responses, or else they stimulate the sympathetic strongly (ether, e.g.) with liberation of cardioaccelerating substances (adrenin, and possibly the liver cardioaccelerator and sympathin). In these experiments it was found advisable to work without anesthesia, by previous pithing of the brain or decerebration, and to ligate the adrenals, but this method involves a long operative procedure and severe operative hazards.

DISCUSSION. The shape of the contractions and relaxations of smooth muscle acted upon by adrenin can, as has been shown, be explained on several hypotheses. It is necessary to refer to other characteristics of the responses to decide which is the probable mode of action of adrenin.

Not all the possible explanations have been considered. Adsorption, for instance, would give a parabolic shape to the curves of the maximal heights of the responses, according to Freundlich's formula. Storm van Leeuwen and Le Heux (1919), who worked on the action of histamine and pilocarpine, adopted this view. The observations on which their curves were constructed are, however, quite inconsistent and they did not analyze the curves mathematically but found in them only a vague analogy to a parabola. If their curves and ours were parabolas the plotting of the logarithms of the responses against the logarithms of the doses of the drug should give a straight line. In fact this plotting gives curves much too different from a straight line to be attributed to experimental error.

Lyon (1923) analyzed the b.p. responses to adrenin and concluded that they obey the Weber-Fechner law, that is, that the rise of b.p. is proportional to the logarithm of the dose of adrenin

$$\left(y = k \log A + k' \text{ or } A = e^{\frac{y - k'}{k}} \right)$$

Such a relation can not be explained by any simple physical or chemical process. But Lyon worked on an unsuitable preparation, since he did not disconnect the heart and vessels from the central nervous system. In our observations the plotting of the responses against the logarithm of the doses of adrenin gives S-shaped curves, which are too different from a straight line to be explained by experimental error; in fact if the error pointed out before (that contraction does not reach its maximum, especially with small doses of adrenalin, because relaxation starts too early) should be considered, the plotting would only be less of a possible straight line than before.

Shackell (1924) studied the responses of isolated arterial rings to adrenin. He reported S-shaped curves which he interpreted as frequency curves due to differences in thresholds of the individual cells of the tissue, around a mean. This interpretation is, however, not admissible, for the contrac-

tions are isotonic and if the responses were in relation to the number of cells contracting a small percentage of active elements would give values very near the maximal shortening, that is, he should *not* get a frequency curve.

Gaddum (1926), who studied the action of adrenin and ergotamine on the excised uterus of the rabbit, offered the same explanation for his results as Shackell did. Here the error in the interpretation lies in the fact that the curves (response: logarithm of concentration of adrenin) are *not* frequency curves, but logarithmic plottings of rectangular hyperbolas. An all-or-none effect in Gaddum's experiments would require differences in threshold of 600 times the concentration first active, and this is improbable. Furthermore, his attitude leads him to contradiction when he attempts to explain the action of ergotamine. After ergotamine he finds the same curve for the action of adrenin, except that higher concentrations are necessary. This he explains as due to the fact that ergotamine has "blocked a fraction of the area in the muscle" on which adrenin must act; this explanation implies that ergotamine has not an all-or-none effect, since he still obtains the same maximal response. In his conclusions he states that the action of ergotamine depends on varying accessibility of the elements of the muscle to the drug, that is, an all-or-none explanation. Gaddum's results can, however, be satisfactorily explained on the chemical hypothesis.

The hypothesis adopted here, that adrenin (A) combines chemically with some substance (H) in the muscle and that the contraction is proportional to the amount of AH formed, which is the same that Hill (*loc. cit.*) postulated for the action of nicotine and curare, is the simplest one that will account for all these experimental facts. One may only speak of probability and may not make conclusive statements because of ignorance of the intermediate stages, i.e., of the nature of the contractile process.

Clark (1926) adopted a similar explanation for the action of acetylcholine on the isolated ventricle and the rectus abdominis of the frog. The formula which he developed to fit his experimental data,

$$Kx = \frac{y}{100 - y}$$

(where y is the action produced in terms of percentage of the maximal action possible and x the molar concentration of the drug) can be readily transformed into the one we have used

$$y = \frac{x}{k' - kx}$$

The logarithmic plottings of his results are quite comparable to ours.

There are not sufficient data at hand to explain adequately the sen-

sitizing actions of previous denervation and of cocaine. Changes of permeability or catalysis might occur. It can, however, be asserted that these agents do not modify the usual mode of action of adrenin.

Our explanation of the mode of action of adrenin excludes an all-or-none response of the smooth muscle when acted upon by the hormone. It has been shown above that Gaddum (*loc. cit.*) could not explain his results on an all-or-none basis. In order to interpret the differences of the responses to varying doses of adrenin as due to differences in the thresholds of the individual cells, it would have to be admitted that the threshold might sometimes be more than 600 times greater for some cells than for others, which is highly improbable. In our experiments both isotonic and isometric contractions produced similar curves (*i.e.*, hyperbolas); had any differences of threshold determined their shape, they would have been frequency curves. It is therefore impossible to reconcile this experimental evidence with the all-or-none principle.

The temperature coefficient, which Hill (*loc. cit.*) used as an extensive argument to support his view, seems to us to have little bearing in this case. The temperature coefficient is clearly that of a chemical reaction, but under the circumstances it is impossible to determine whether it is the excitatory or the contractile process which is involved.

The hyperbolic shape of the curves of maximal height of the responses plotted against the doses of adrenin suggests some practical consequences. In biological quantitations of adrenin, whatever the method employed, it is convenient to use dilutions of the unknown solution to be tested which will give responses in the ascending part of the hyperbola, for the readings will then be more precise. More concentrated solutions will give points very close to the asymptote with very slight differences between relatively large differences in concentration. In a given preparation it is sufficient to determine two points of the curve with a known standard to obtain the values of k and k' . It will afterwards not be necessary to match the height given by the unknown to find out the amount of adrenin it contains, as this amount can be readily figured from the formula. This calculation simplifies the technique.

The usual variations of blood pressure or heart rate in an organism occupy points placed in the ascending portion of the hyperbola. This means that injections of adrenin in order to raise blood pressure may readily exceed their object and, if the circulatory system is damaged, may cause a dangerous hypertension. The minimal adequate dose is a very precise one and neighboring doses will be insufficient or excessive. With hypodermic injections absorption is uncertain and uncontrollable. The only correct method of administering adrenin to reestablish normal heart rate or blood pressure is the intravenous injection at an adequate rate, controlled constantly by examination of the results.

SUMMARY AND CONCLUSIONS

The responses of smooth muscle to adrenin obey the equations $y = k(1 - e^{-\lambda t})$ for the contraction and $y = ke^{-\lambda t}$ for the relaxation. This shape of the responses may be explained on either a physical or a chemical hypothesis.

The chemical hypothesis, according to which adrenin (A) would combine with an unknown substance (H) in the muscle and the contraction would be proportional to the amount of (AH) formed, requires that the plotting of the maximal height of the responses against the doses of adrenin should have the shape of a rectangular hyperbola with asymptotes parallel to the axes. The physical hypotheses examined (diffusion and adsorption) lead to different shapes of this curve.

The analysis of the curves obtained by plotting in this manner the response of the nictitating membrane (previously or acutely denervated, without or after injecting cocaine), of the blood pressure (with or without cocaine), of the rate of the heart (previously or acutely denervated), and of the non-pregnant uterus of the cat, to single injections of adrenin (full dose in 1 cc. in 5 seconds) or to longer injections (several minutes) at a constant rate, shows that these curves are rectangular hyperbolas with asymptotes parallel to the axes, as the chemical hypothesis requires.

The temperature coefficient (μ in Arrhenius' formula) of the responses of the nictitating membrane to adrenin is approximately 30,000, adequate for a chemical reaction and too high for a physical process.

It is therefore concluded that the chemical hypothesis accounts for the results observed, whereas the physical hypotheses mentioned do not.

The sensitized nictitating membrane and Elliott's blood-pressure preparation are compared as quantitative indicators of adrenin, and the nictitating membrane is found equally sensitive, equally non-specific, more accurate, simpler to prepare and equally lasting.

A simplification of all biological methods for the quantitative determination of adrenin is suggested by determining the constants of the hyperbola of the preparation with only two injections of the standard. The amounts of adrenin contained in the unknown solution may then be readily calculated.

It is shown that the chemical hypothesis excludes an all-or-none response of smooth muscle to adrenin. The all-or-none interpretation is discussed. It is concluded that it cannot be reconciled with this experimental evidence.

In conclusion I wish to express my thanks to Dr. M. Vallarta, of the Massachusetts Institute of Technology, for his help in the mathematical aspect of the problem, and to Dr. H. Davis for valuable suggestions and criticism.

BIBLIOGRAPHY

- BARGER, G. AND H. H. DALE. 1910. *Journ. Physiol.*, xli, 19.
BAYLISS, W. M. 1915. *Principles of general physiology*. London, 724.
CLARK, A. J. 1926. *Journ. Physiol.*, lxi, 530.
ELLIOTT, T. R. 1912. *Journ. Physiol.*, xliv, 374.
GADDUM, J. H. 1926. *Journ. Physiol.*, lxi, 141.
HILL, A. V. 1910. *Journ. Physiol.*, xxxix, 361.
LANGLEY, J. N. 1921. *The autonomic nervous system*. Cambridge, 44.
LYON, M. 1923. *Journ. Pharm. Exp. Therap.*, xxi, 229.
ROSENBLUETH, A. 1931. *This Journal*, xcviii, 186.
1932. *Ibid.*, c, 443.
ROSENBLUETH, A. AND W. B. CANNON. 1932. *Ibid.*, xcix, 398.
SHACKELL, L. F. 1924. *Journ. Pharm. Exp. Therap.*, xxiv, 53.
STORM VAN LEEUWEN, W. AND J. W. LE HEUX. 1919. *Pflüger's Arch.*, clxxvii, 250.
TRENDELENBURG, P. 1924. In *Handbuch d. Exp. Pharmak.*, Berlin, 1130.

GLUCOSE TOLERANCE IN THE PHLORHIZINIZED DOG AS AFFECTED BY RENAL VESSEL LIGATION AND BY INSULIN

L. A. GOLDSTEIN, A. J. TATELBAUM, S. EHRE AND J. R. MURLIN

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

The purpose of the experiments described in this paper was to secure additional information regarding the effects of phlorhizin on the utilization of glucose and, incidentally, to determine whether insulin can be shown to restore to any noteworthy degree the power of the kidney tubules to reabsorb sugar.

A rate of fall in the tolerance curve after ligation in the phlorhizinized animal as great as, or greater than, before ligation would indicate that there is no obstacle to the temporary disposal of the sugar at least. For, blocking one avenue of escape (the kidney) should, if there were serious impairment of permeability to the tissues, or of utilization therein, disclose this impairment by a retardation in the recovery to the pre-injection level. Further, should the rate of fall following ligation in the fully phlorhizinized dog approximate the rate of fall in the normal dog under identical conditions of dosage and rate of infusion, it would follow that removal of the kidneys from the circulation restores the animal to normal so far as withdrawal of sugar from the circulation is concerned. It would be difficult to reconcile such a finding with the view expressed by Nash and Benedict (1) that phlorhizin "produces an intrinsic impairment of the sugar-burning mechanism," or the view expressed by Gaebler and Murlin (2) that phlorhizin may inactivate insulin in the tissues.

The evidence against these views from the standpoint of combustion seems to be nearly¹ complete in the work of Wierzuchowski (3) and Deuel, Wilson and Milhorat (4), but from the standpoint of permeability of the tissues the evidence is conflicting.

It would be very strange if phlorhizin could so completely destroy the power of the kidney tubules to reabsorb sugar without at the same time affecting the power of any other cells to absorb the same sugar. Moreover, there seems to be no evidence that the effect of phlorhizin is on the

¹ We are obliged to say *nearly* because it has not yet been shown that phlorhizin does not delay the secretion of insulin, nor that the improved tolerance after carbohydrate in the phlorhizinized, previously fasting dog is exactly the same as its improvement in simple starvation.

tubular cell exclusively and not also on the endothelial cells of the capillaries which also must be passed by the reabsorbed sugar in the normal kidney. It might conceivably injure the power of the endothelial cells to absorb sugar *into* the blood and not affect the power of similar cells elsewhere to pass sugar *from* the blood. But this again would be an extraordinary degree of specificity. A brief review of the investigations which have touched this general phase of the subject, whether consciously or not on the part of the authors, would seem to be necessary.

Minkowski (5) extirpated the kidneys from four dogs, two of them previously made diabetic with phlorhizin and two by excision of the pancreas. The blood sugar rose only slightly in the former, but rose approximately to double the already high level in the latter. His explanation (p. 148 *et seq.*) is that by extirpation of the pancreas the power to consume sugar is destroyed directly, a conception which, if we include glycogen formation as well as combustion in the term consumption of sugar, has been fully confirmed by the recent work on insulin. In consequence, he says, the heaping up of sugar must increase still further after cessation of urine secretion. In contrast, he points out that phlorhizin causes a passage of sugar into the urine without directly disturbing the consumption of sugar. Phlorhizin diabetes therefore leads at once to impoverishment of the blood. When the renal function drops out the sugar content can become normal again. But an immoderate accumulation does not occur because the destruction of sugar is not disturbed. Minkowski makes it clear that the slight rise in blood sugar which followed double nephrectomy in the phlorhizinized animal came entirely within the normal range. There was no interference with the escape of sugar once it reached the normal level.

Underhill (6) also used the method of renal exclusion as a means of testing the capacity of the phlorhizinized animal to dispose of sugar. He criticised Minkowski's work as not having supplied in the phlorhizinized dogs a continuous condition comparable with the defect produced by extirpation of the pancreas. He believed it was necessary to establish the animal in a state of total diabetes and took the Lusk D:N ratio of 3.65 as his criterion. Two dogs brought into this condition by administration of phlorhizin for three days were operated under ether anesthesia by double ligation of the renal vessels in such a way as completely to stop excretion of urine. Blood from the femoral artery without anesthesia showed blood sugar values approximately two and three times the normal in the two dogs at 6 and 7½ hours respectively after ligation.

Pearce (7) found small amounts of sugar in pancreatic, gastric and salivary juices of dogs rendered glycosuric with phlorhizin which he thought demonstrated an increased permeability of these glands to sugar.

Csonka (8) gave 50 grams glucose per os to two dogs, one normal the other phlorhizinized, subsequent to ligation of the renal vessels and ureters. The phlorhizinized dog showed marked hyperglycemia, the normal dog practically none. The author does not offer any explanation.

Deuel, Wilson and Milhorat (4) employed nephrectomized dogs in one division of their study of the mechanism of phlorhizin diabetes in order to test more severely the possibility of a sudden flushing out of glycogen when phlorhizin was given. Since no hyperglycemia resulted up to 12 hours following administration, either in the normal dog or in the dog whose kidneys had been removed, they concluded that "phlorhizin *per se* does not cause any breakdown of the glycogen reserves." These experiments were not, strictly speaking, tolerance tests; but it may be stated with

considerable confidence that if phlorhizin had altered the permeability of the tissues for sugar, particularly if it had decreased the permeability materially, their result scarcely could have been the same. In a later paper Deuel (9) made comparisons of the glucose tolerance of normal and phlorhizinized dogs following a definite dose of the sugar given by stomach tube to the same animal, on the one hand while fasting and on the other 14 hours after a generous carbohydrate feeding. The tolerance was much greater (curve lower) in the animal whose carbohydrate reserve had been built up than in the one whose reserves had been depleted; but for our present purpose a more important result was that the phlorhizinized animal showed essentially the same tolerance as the normal in both conditions.

Deuel and Gulick (10) gave ammonium chloride to reduce the alkali reserve of some of the normal dogs used by Deuel (9) but, notwithstanding a considerable reduction of the CO_2 -combining power, there was no diminution of tolerance to glucose by mouth. Acidosis, therefore, is not always a cause of reduced tolerance.

Nash (11) studied the effects on glycogen storage of ligating the ureters in phlorhizinized dogs and found no evidence of diminished utilization in these dogs compared with others not phlorhizinized. Exclusion of the renal function in this manner caused the blood sugar of the glycosuric animal to behave as in the non-glycosuric animal. Ketosis disappeared within a few hours after ligation in the phlorhizin-treated animal, showing that the sugar raised to the normal threshold was able to penetrate the tissues readily.

Thus Deuel and his associates and Nash furnish evidence in support of Minkowski's original conception while the few observations of Underhill and Csonka weigh something against it. The work of Pearce could be interpreted as favoring tolerance in the sense of facilitating the passage of sugar from the circulation. Since acidosis cannot be looked upon as the cause of diminished tolerance (10) in the work of Underhill and of Csonka and since carbohydrate feeding in Deuel's work did improve the tolerance, while the injection of insulin also improves the tolerance (Nash (11)) it may be inferred that the presence of insulin in the tissues in plentiful amount explains the results where no loss of tolerance has been noted and the lack² of it explains the results where there was a significant loss. At all events this is the hypothesis with which the work here reported was undertaken.

A standard procedure was adopted consisting, briefly, of the use of amytal in definite dosage, a definite dose of glucose and, when used, also of insulin, per unit of weight, both of the latter given intravenously and together. The choice of the amytalized dog for study of the tolerance curve has its justification in the necessity for some sort of anesthesia in ligation of the renal vessels. Since it was desirable to study the effects of ligation immediately, before accumulation of waste products could bring about any considerable change of tolerance (12, 13), the other tolerance curves likewise were obtained under amytal. We are well aware that several observers have reported that amytal alters the carbohydrate

² Neither Underhill nor Csonka gives any information regarding the nutritive condition of his animals.

metabolism. Hines, Boyd and Leese (14) found a higher hyperglycemia from a constant rate of intravenous sugar injection, than in the unanesthetized animal and a consequent more rapid excretion into the urine; and they infer an impairment of the glycogenetic function, presumably in the liver. Wierzuchowski and Gadowska (15) also reported increased glycosuria during continuous injection of glucose and decreased assimilation velocity. Olmsted and Giragossintz (16) observed that the drug caused spasm of the pyloric sphincter and diminished absorption from the duodenum. In the experience of this laboratory (17) dogs may be kept under amytal for many hours at a time without any fluctuation of blood sugar beyond the limits of accuracy of the method. This was confirmed during the present study. It is important not to use so much alkali as to cause marked slowing of the respirations, for partial asphyxia results and this alone is sufficient to cause hyperglycemia.³

The intravenous injection of glucose was chosen because it does away with the irregularities of absorption (18), because it is better tolerated by phlorhizinized animals (19) and because successive tolerances are more likely to duplicate each other (20). It is important, however, that the dose chosen for successive tolerances should be of very moderate size (21). In these experiments the dose was 0.5 gram per kilogram of body weight and the rate of injection 3.75 grams per minute (see protocols below).

EXPERIMENTAL. Ten dogs were used in this investigation, three of which were normal controls and seven were phlorhizinized by the Coolen method. The procedures were very similar in all the latter cases. All the dogs were fed 500 grams ground beef (hamburg steak) daily at 4 to 5 o'clock p.m. until the day of experiment when no food was given. Illustrative protocols of the three types of experiments are given below.

PROTOCOLS. Dog 3. Normal. Weight 18.4 kgm. November 19, 1929
8:00 a.m. Catheterized. Urine sample negative. Benedict qualitative

³ Just as this paper is going to press some experiments have been completed in this laboratory by Messrs. W. Dusselman, Jr. and M. R. Hosie, on the effects of sodium amytal upon the glucose tolerance curve of a normal dog and the same dog after phlorhizin. These experiments indicate a difference in the normal dog which is probably to be explained entirely by the effect of the first tolerance dose (before amytal) on the second tolerance curve (first after amytal). A second tolerance after amytal, at an hour's greater interval, was very similar both as regards peak effect and rate of recession (see p. 172) to the normal tolerance curve. When the dog had been under phlorhizin treatment for three days, a dose of epinephrin having been given with the last dose of phlorhizin, a D:N ratio of 3.6 was obtained. The next day the tolerance tests were made and the first after amytal showed a somewhat higher peak effect, but the same general rate of recession, up to 2 hours and 40 minutes, as the test before amytal. We are indebted to Messrs. Dusselman and Hosie for permission to mention these results. They indicate very slight, if any, alteration from normal in the capacity of the tissues to remove glucose from the blood, in the amytalized animal, whether previously phlorhizinized or not.

8:30 a.m. Injected 33 cc. amytal subcutaneously

10:05 a.m. Normal blood sample. Blood sugar 100 mgm. per cent

10:12 to 10:16:30. Injection of 18.5 cc. of 50 per cent glucose (1 cc. per kilo) and rate equaled 1 cc. per 8 seconds

SAMPLE NUMBER	TIME	MINUTES AFTER INJECTION	BLOOD SUGAR	REMARKS
	<i>a.m.</i>		<i>mgm. per cent</i>	
1	10:22	6	342	—
2	10:29	13	190	—
3	10:39	23	120	—
4	10:49	33	85	—
5	10:59	43	66	—
6	11:09	53	69	—
7	11:16	60	63	—
8	11:36	80	75	—
9	11:56	100	84	—
10	12:16	120	77	Respirations stopped; dog cyanotic; died soon after

12:27 p.m. Catheterized

Urine—positive Benedict on cooling

Dog 4. Weight 18.0 kgm. Phlorhization

Rations—500 grams ground meat

Saturday, November 16, 1929, 4:00 p.m. injected subcutaneously 1 gram phlorhizin in 10 cc. olive oil

Monday, November 18, 1929, 4:00 p.m., injection 1 gram phlorhizin in 10 cc. olive oil

Tuesday, November 19, 1929, 3:30 p.m., injection and started collection of 24-hour urine sample. Also injected 1 cc. 1 to 1000 adrenalin

Wednesday, November 20, 1929, 4:30 p.m., injection

Urine analysis: Volume (24 hours) 1560 cc., urine sugar (Hawkins and Van Slyke (1929)) 1 to 99.06 grams, urine N 28.8 grams, D:N equals 3.44.

Dog 4. Phlorhizinized. November 21, 1929, (A) Kidneys intact

8:15 a.m. Injected 32.4 cc. amytal subcutaneously

10:37 a.m. Basal blood sample, before injection, 35 mgm. per cent

10:45 to 10:48 injected 18 cc. 50 per cent glucose solution (1 cc. per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				<i>mgm. per cent</i>
1	10:54	Left jugular vein	6	213
2	11:01	Left jugular vein	13	103
3	11:10	Left jugular vein	22	88
4	11:20	Left jugular vein	32	70
5	11:30	Left jugular vein	42	62
6	11:40	Left jugular vein	52	64
7	11:48	Left jugular vein	60	54
8	11:58	Left jugular vein	70	54
9	12:08	Left jugular vein	80	47
10	12:29	Left jugular vein	101	43
11	12:55	Left jugular vein	127	40

Dog 4. Phlorhizinized. November 21, 1929 (B) Renal vessels ligated
 1:30 to 2:30 p.m. Vessels of both kidneys ligated
 2:35 p.m. Basal blood sample, before injection, 74 mgm. per cent
 2:45 to 2:48. Injected 18 cc. 50 per cent glucose solution (1 cc. per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR <i>mgm. per cent</i>
1	2:54	Right jugular vein	6	392
2	3:01	Right jugular vein	13	231
3	3:13	Right jugular vein	25	180
4	3:20	Right jugular vein	32	147
5	3:37	Right jugular vein	49	135
6	3:40	Left saphenous vein	52	134
7	3:49	Left saphenous vein	61	119
8	4:08	Right femoral vein	80	110
9	4:28	Right femoral vein	100	95

Autopsy at 6:15 showed that the renal vessels were securely tied. Dog died soon after last blood sample.

Dog 5. Phlorhization

500 grams ground beef daily

November 19, 1929, 3:30 p.m. Injected 1 gram phlorhizin in 10 cc. olive oil
 November 20, 1929, 4:30 p.m. Injected 1 gram phlorhizin in 10 cc. olive oil
 November 21, 1929, 5:00 p.m. Same as above, also 1 cc. 1 to 1000 adrenalin. Also started 24-hour urine samples. At 10:00 p.m. 1 cc. 1 to 1000 adrenalin
 November 22, 1929, 8:00 a.m. Injected 1 cc. 1 to 1000 adrenalin 4:30 a.m. Injected phlorhizin and adrenalin

Dog 5. Phlorhizinized. November 23, 1929 (A) Before insulin
 7:45 a.m. Injected 15 cc. amytal intravenously
 8:00 a.m. Basal blood sample (Rt. jugular vein) before injection 37.4 mgm. per cent
 8:03 to 8:06. Injected 16 cc. 50 per cent glucose solution

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR <i>mgm. per cent</i>
1	8:12	Right jugular vein	6	174
2	8:19	Left jugular vein	13	137
3	8:28	Left jugular vein	22	118
4	8:38	Left jugular vein	32	105
5	8:48	Left jugular vein	42	92
6	8:58	Left jugular vein	52	79
7	9:12	Left jugular vein	66	63
8	9:26	Left jugular vein	80	69
9	9:46	Left jugular vein	100	52
10	10:06	Left jugular vein	120	51
11	10:34	Left jugular vein	148	54
12	10:55	Left jugular vein	169	47

Dog 5. Phlorhizinized (insulin). Weight 16 kgm., November 23, 1929, (B) After insulin
 10:55 a.m. Basal blood sample, before injection, 48 mgm. per cent

10:50 to 11:01. Injection of 16 cc. 50 per cent glucose solution plus 16 units insulin (1 cc. glucose per kgm.; 1 unit insulin per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				<i>mgm. per cent</i>
1	11:07	Left jugular vein	6	123
2	11:14	Left jugular vein	13	87
3	11:23	Left jugular vein	22	52
4	11:33	Left jugular vein	32	43
5	11:43	Left jugular vein	42	33
6	11:53	Left jugular vein	52	25
7	12:01	Left jugular vein	60	24
8	12:11	Left jugular vein	70	23
9	12:21	Left jugular vein	80	30
10	12:41	Left jugular vein	100	39
11	11:01	Left jugular vein	120	19
12	1:28	Left jugular vein	147	20

Dog 5. Phlorhizinized (insulin) November 23, 1929, (C) Renal vessels ligated
 1:20 p.m. to 2:00 p.m. Renal vessels (both kidneys) ligated
 2:08 p.m. Basal blood sample, before injection, 28 mgm. per cent
 2:22 to 2:25 p.m. Injected 16 cc. glucose plus 16 units insulin (1 unit insulin per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				<i>mgm. per cent</i>
1	2:31	Left femoral vein	6	112
2	2:38	Left femoral vein	13	67
3	2:47	Left femoral vein	22	56
4	2:57	Left femoral vein	32	37
5	3:07	Left femoral vein	42	31
6	3:17	Left femoral vein	52	29
7	3:25	Left femoral vein	60	22
8	3:35	Left femoral vein	70	23
9	3:45	Left femoral vein	80	Lost
10	4:05	Left femoral vein	100	17
11	4:25	Left femoral vein	120	15
12	4:52	Left femoral vein	147	14

RESULTS. The principal results of this study are contained in the charts. It seems necessary, in the interest of brevity, to introduce a few terms with well defined meanings. By "peak effect" is meant the rise of blood sugar in milligrams per cent from the preinjection level to the level which it reaches at 6 minutes following completion of the injection. This point was chosen after some preliminary experiments designed to locate the point of maximum concentration. It is not offered as a fixed topographic feature of the tolerance curve for all circumstances of injection. Obviously such a feature must depend upon amount of sugar injected and

the rate. Nothing more is claimed than that this point (six minutes) comes very close to the maximum under the conditions chosen, and is a resultant of several factors made as nearly uniform as possible, and common to all the experiments. Any marked change in level at this moment then should be explainable by the superimposed factor.

By "rate of recession" is meant the total time and amount of fall from the peak to the preinjection level of the blood sugar. Another way of measuring the rate of disposal of the sugar is to calculate the total fall from peak to a particular time, and attention will be called to differences with respect to this criterion also. For the latter purpose we have found it most convenient to limit the comparison to 42 minutes after completion of injection or 36 minutes after the peak, although most of the curves were followed to at least two hours, and many of them longer.

The term "slope of return" or "downward slope of the curve" cannot well be used synonymously with "rate of recession;" for, as we shall see,

TABLE 1

CONDITION	DAY OF PHLOR- HIZIN	DOG NUMBER	WEIGHT	PEAK EFFECT	RATE OF RECESSION	
					Time	Fall
			kgm.	mgm. per cent	min.	mgm. per cent
Normal.....		3	18.4	242	28	242
Normal.....		10	11.2	180	25	168
Phlorhizin.....	5	4	18.0	178	127+	162
Phlorhizin.....	4	5	16.0	137	169+	139
Phlorhizin.....	3	9	13.0	155	145+	161

the downward limb is really biphasic. Quite possibly the two phases have somewhat different meanings. Certainly they have different rates. Nevertheless the curve as a whole has a rate of descent if we limit it to a certain terminal level, and it is this total rate which is meant by rate of recession. These two criteria, peak and rate of recession, are usually sufficient by which to judge the ease of escape of the sugar from the circulation, which is what we really mean by tolerance. The chief concern in this study has been to analyse the features of the tolerance curves obtained under conditions very nearly uniform to see, if possible, what factors may be explained by them.

Dealing with one factor at a time we shall present the results in the following order: 1, effect of phlorhizin on the intravenous glucose tolerance; 2, the effect of renal vessel ligation in restoring the tolerance; and 3, the effect of insulin.

1. *Effect of phlorhizin on tolerance.* In the gross this effect can be seen by a glance at table 1. All the tests contained in this table are first

tolerance tests, two on normal and three on phlorhizinized dogs before ligation was imposed or insulin given, all dogs under amytal.

It is evident that the normal dog is characterized by a quick return of the blood sugar to its original concentration, and the phlorhizinized dog by a very slow return. As a matter of fact, none of the latter accomplished the complete return to the preinjection level in the time allotted. This fact is indicated by the plus signs after the numbers for minutes. In addition to the more rapid rate of recession the normal dog also shows a higher peak effect. This is readily understood. The kidney leak after phlorhizin prevents the concentration from reaching the high normal level.

The rate of recession is not so simple a matter. Merely measuring the extremes of the curves or laying them off on coördinate paper, the rate of discharge from the blood seems to be so much lower in the phlorhizin animal as to leave but one conclusion possible; namely, since the escape by way of the kidney is so much more rapid,⁴ the escape by all other channels (penetration into the tissues for glycogen formation, combustion, possibly fat formation) must be very much below the normal. Critically examined, however, the curves in figure 1 show clearly that the rate of recession is biphasic, and it is necessary to consider the two phases separately. The embarrassment comes in saying just where the division point occurs. Several will be considered.

The curves are most divergent during the early part of their descent, say for the first 30 minutes. At about this time they begin to run similar courses, and at 40 minutes, with one exception (dog 5), they are so close together, both as regards height and slope, as to be considered, for all practical purposes, identical. They continue thus until 60 minutes, but from this time on they diverge somewhat. An arbitrary level, say 100 mgm. per cent, may be taken as the dividing point. They are widely scattered at this point and 100 mgm. per cent for total reducing substance is not far from the normal level. The question then for the first phase would be, how long does it require the phlorhizinized dog, in comparison with the normal, to restore the normal level? Dividing the peak elevation above normal (100) by the number of minutes after injection necessary to reach that level again, we get values which (except for dog 4) are roughly $\frac{1}{30}$ of the elevation. In other words, the fall is $\frac{1}{30}$ of the height per minute. Specifically, 240 mgm. per cent of "head" produces a fall to normal at the rate of 8 mgm. per minute and 75 mgm. per cent produces the fall to normal at the rate of 2.5 mgm. per minute. The former is a normal dog (no. 3) and the latter a phlorhizin dog (no. 5). The first phase appears then to be determined principally by the "head" of concentration. One would naturally expect that the kidney lesion would produce

⁴ Sugar was not estimated in the urine.

a much more rapid fall in the phlorhizinized dog, and this seems to be true of dog 4, which returned to the normal level in 6 minutes. But for the group as a whole the dominant factor seems to be the "head" of concen-

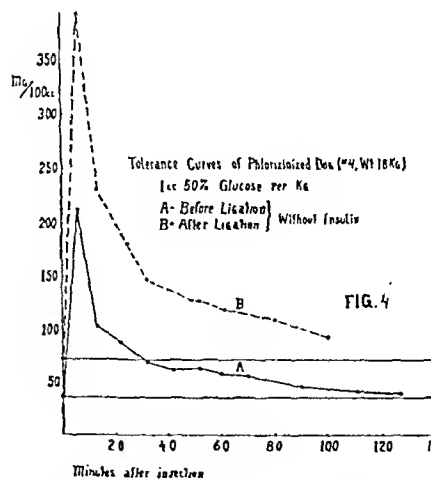
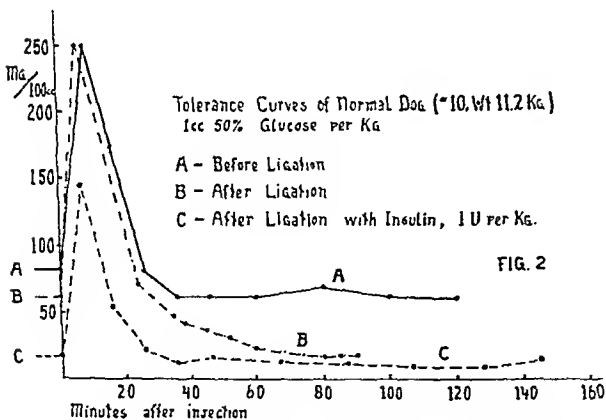
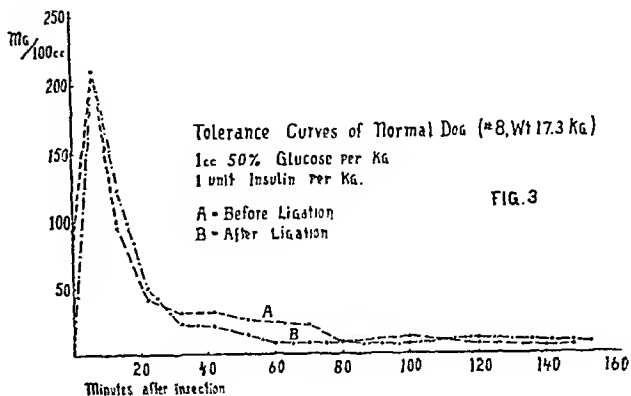
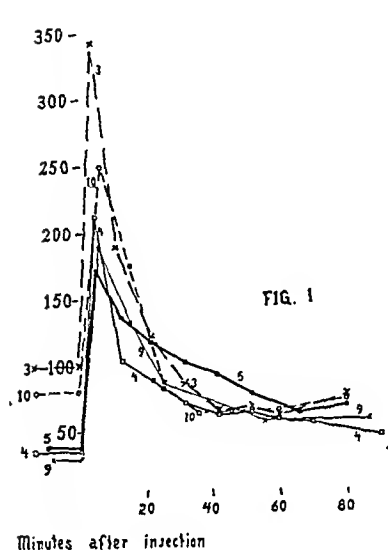


Chart 1. Glucose tolerance curves of dogs anesthetized with sodium amytal. Fig. 1, smooth curve, dogs under phlorhizin for at least 3 days before anesthesia; broken line, normal dogs. Further data for these curves are given in table 1. Figs. 2, 3 and 4, all curves in each case are taken successively on same dog. Further explanation in chart and in text.

tration, regardless of whether the animal has been poisoned with phlorhizin or not. Granting this, and knowing that the kidney leak is present in all the phlorhizinized dogs, it follows that other channels of escape must be blocked to some extent.

If the normal level be taken as the beginning of the *second phase* the advantage is gained that the "head" at the starting point is equalized and therefore is ruled out as a determinant of the slope of direction for the remainder of the curve; also it may serve to contrast more clearly the different factors which serve to carry the curve below normal. There is good reason for believing that the fall of the tolerance curve below normal for a well-nourished animal or man is due to the production of insulin stimulated by the hyperglycemia (Foster, 22). In the phlorhizin glycosuric animal this cannot be the only factor and therefore, if it is a factor at all (and it need not be), the production of insulin must be at a slower rate than in the normal. If phlorhizin produced a diminished rate of penetration to the tissues, the fall below normal would be a resultant of this and the kidney leak, or possibly of the increased production of insulin in addition to these two. It is evident from figure 1 that the rate of fall from 100 mgm. to about 60 mgm. is considerably slower in all the phlorhizinized dogs than in the normal. This feature will be discussed later.

The relative order of the facts for the second phase would be the same, if instead of the normal level of blood sugar the first points of distinct deflection in the direction of the curves were taken as the division point between first and second phase. The difficulty with this criterion however is that the "head" of concentration remains as a factor and, secondly, it is often very difficult to say where the point of distinct deflection occurs, for the change of direction is quite gradual.

There is also the point at which the curve reaches the horizontal, meaning of course that the inflow to the blood is just equal to the outflow. This criterion has the advantage that equilibrium has been reached and, particularly after the renal vessels have been ligated, it is a useful criterion, as we shall see.

Probably the dogs of the group we have been comparing, two normal and three phlorhizinized, did not have perfectly equal glycogen stores, when the first tolerance tests were made. Indeed we doubt whether the two normals were exactly equal in this respect. The dogs were fed nothing but meat and the feedings were regular, but none was fed on the day of experiment and it is practically certain that the glycosuria in addition to fasting for 18 to 20 hours reduced the glycogen of the phlorhizinized animals more than fasting alone, for an equal length of time, reduced it in the normals. The greater need for glycogen should cause more rapid withdrawal of sugar from the circulation and this need has often been cited as an explanation of the lack of combustion of sugar when fed after a fasting period. The work of Wierzuchowski and of Deuel, Wilson and Milhorat makes it clear, however, that pre-feeding with carbohydrate facilitates both the withdrawal of sugar from the circulation and its combustion. Deuel believes this is best explained by the production of more insulin.

But as we have just seen, the production of insulin scarcely can be as great after phlorhizin as before.

The question which now arose was, whether the *administration of insulin* would affect the tolerance in the same way as the administration of carbohydrate. We shall be ready for this question when we have considered the effects of ligation.

2. *Ligation of the renal vessels.* Ligation in the normal dog produces no effect on the absolute level of the peak (fig. 2). Since, however, the first tolerance carries the blood sugar below normal, it does produce a greater "peak effect" according to the definition given (p. 172). When insulin is given and sufficient time is not permitted for complete recovery from the first tolerance before the second is given (fig. 3) the same is true, only the peak effect, i.e., from preinjection level, is accentuated. Ligation itself produces no change in the rate of recession. It appears to do so in figure 2. The curve A obtained before ligation reaches the preinjection level at about the same time as curve B, obtained after ligation; but A strikes the horizontal long before B and it appears that ligation has affected the time necessary for equilibrium to be established. This, however, is better explained by the supposition that the first tolerance dose was not sufficient in this dog to stimulate the production of insulin, whereas the second dose, from the cumulative effect, was sufficient and as a consequence the curve was carried far below the normal level. With dog 8, where certainly there must have been sufficient insulin, the disappearance of sugar, from a given "load," was not affected (fig. 3).

Ligation in the phlorhizinized dog without added insulin produces the change which we should expect (fig. 4). The peak effect is even greater than in any normal animal studied in this series (see fig. 1). Also the rate of recession in the first phase is greater than that of the normal, suggesting that "head" of concentration is the determining factor just as it appeared to be in the normal and while the kidney leak was present. It will be noted, however, that there would be no *second phase* if the normal level (100 mgm.) were adhered to as the starting point. Before ligation this level was reached in about 22 minutes; after ligation it had not been reached in 100 minutes. If the point of deflection be taken as the starting point, it is seen to occur at about the same time following the peak, after as before ligation, and at about the same relative position (slightly higher) on the descending limb. From this point the general form of the curve seems to be very nearly the same after as before ligation. In other words, the chief difference between the two curves is one of level. Ligation has elevated the curve throughout its course. Actually the whole second phase, from this point of deflection, is approximately twice as steep after ligation as before, falling 135 mgm. per cent in 85 minutes for curve B, as compared with 60 mgm. in the same time for curve A. Eliminating

the kidney leak has raised the peak effect to approximately double, indicating lessened tolerance (because the peak is higher than for any normal dog) but it has also increased the rate of recession (second phase) to approximately double, indicating better utilization.

We do not believe that the point of deflection should be chosen, because the effect of "head" is not eliminated. Moreover, it must be borne in mind that the curve following ligation describes a second tolerance on the same animal and that the first dose of sugar probably has affected the second tolerance to some extent, either by stimulating the production of insulin or by affecting in some other respect the capacity of the tissues to take up and hold sugar. It is the more striking therefore that the ligation has not restored the tolerance curve completely to normal.

Comparison with the normal tolerance curve in figures 1 and 2 shows clearly that as regards *total rate of recession* the restoration is not complete. For example, normal dog 3 (fig. 1) from a peak nearly as high, recovered the preinjection level in 28 minutes; normal dog 10 (fig. 2) recovered in 25 minutes. Phlorhizinized dog 4 *after ligation* had not reached this level in 100 minutes, and required 90 minutes to reach the normal level of 100 mgm. per cent.

But there is another feature of the normal curve which is not restored by ligation in the phlorhizinized dog; namely, the quick change of direction to the horizontal or, in other words, to equilibrium. Dog 3 (normal) reached temporary equilibrium at least in 40 minutes; dog 10 (normal) before ligation in approximately 60 minutes. Dog 4 (phlorhizin) after ligation had not reached it in 100 minutes.

This failure to reach equilibrium after the kidney leak had been removed may be explained by the fact that the first tolerance dose was not sufficient (it was wasted largely through the kidney) to restore the tissue content of sugar, so that instead of leaking out by the kidney the blood sugar after ligation was still "leaking" out to the tissues at 100 minutes. The "leak" to the tissues is at a greater rate, however, on account of greater "head" than the leak through the kidney. Thus far our analysis confirms in two respects the results of Underhill and Csonka, and in one the work of Minkowski.

3. *Effect of insulin on tolerance before and after ligation.* When the same experiment as that on dog 4, just discussed, is carried out on the phlorhizinized dog in exactly the same manner, except that insulin is given with the tolerance dose of glucose both before and after ligation, the peak difference is not so great (dog 6, fig. 5) and the rate of recession is greater both before and after ligation, as we should expect. In absolute terms the fall from peak is not so great. Dog 6 before ligation in 42 minutes recovered from peak 175 mgm. to 35—a decline of 140 mgm. After ligation the recovery in the same time was 170 mgm. (compare with figures for dog 4 on

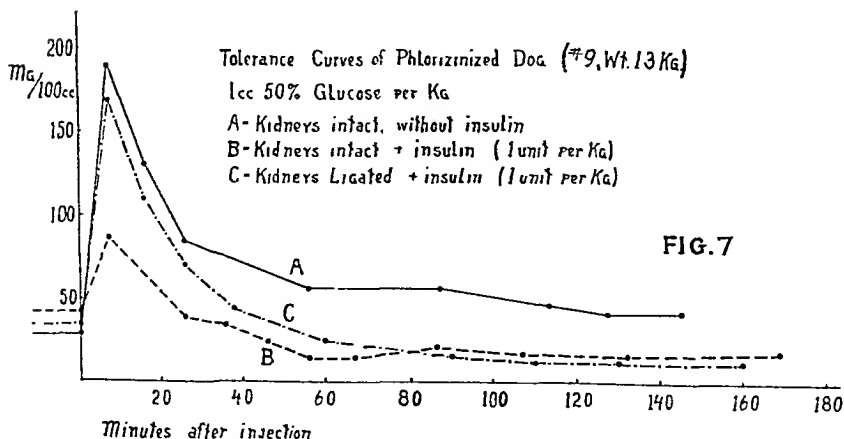
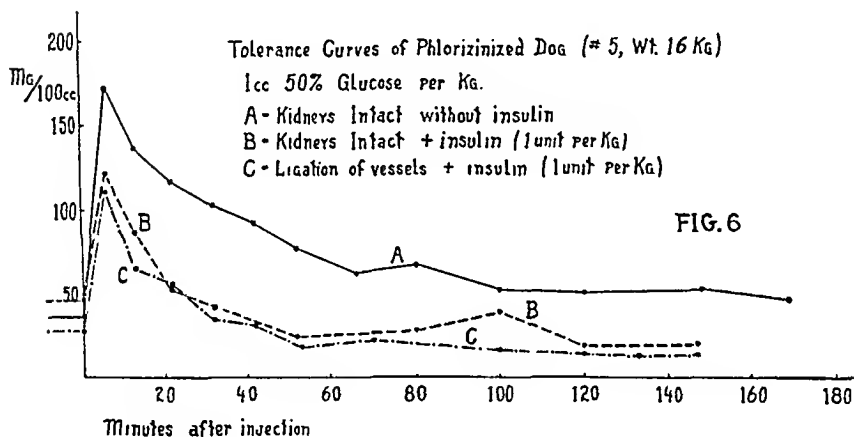
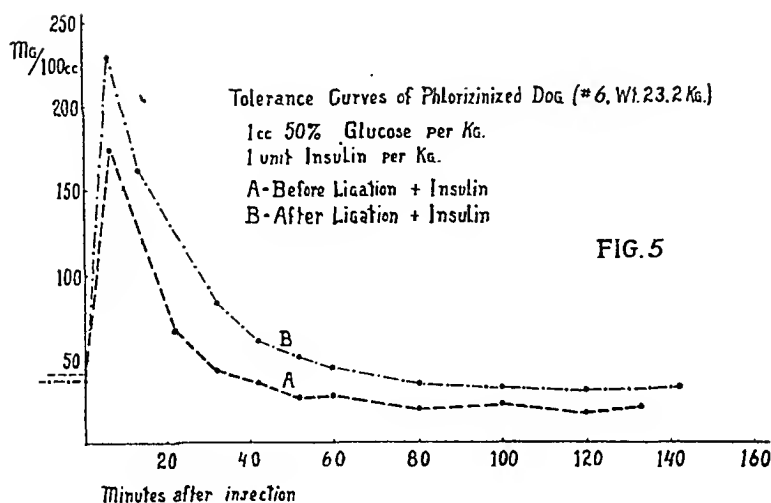


Chart 2. Glucose tolerance curves on anaesthetized, phlorhizinized dogs, before and after renal vessel ligation; with and without insulin. All curves in each figure are taken successively on same dog.

p. 177). Equilibrium was reached in the two curves in 50 and 80 minutes respectively. This is not so rapid as in the normal dog without extra insulin. It seems to require something more than ligation and extra insulin to restore all features of the normal tolerance curve. If a previous feeding of carbohydrate restores the normal tolerance curve, as Deuel believes, because it stimulates the production of insulin, it must produce more, or at least what is produced acts more effectively, than 1 U. per kgm.

With dogs 5 and 9 (figs. 6 and 7) three successive tolerances were performed. Curve *A* in each case was obtained before ligation and without insulin. These curves have already been discussed in figure 1. Curve *B* was obtained before ligation but with insulin, and curve *C* after ligation with the same dose of sugar and insulin. In neither case is the restoration to the normal curve quite complete in all respects. The rate of recession, as specifically defined on page 172 is just as great, but the time necessary to reach equilibrium remains somewhat greater than the normal.

The differences between figures 6 and 7 do not invalidate the comparison. Curve *C* in figure 6 is slightly lower (peak) than curve *B*, while in figure 7 it is nearly as high as *A*. In the former case the third tolerance was started earlier, before the previous insulin dose had worn off so completely. Hence the insulin of the third tolerance produced a greater effect on the peak than ligation, while with dog 9, ligation produced a greater effect than the insulin given afterward.

It is not the intention to lay too great a stress upon the failure of ligation and insulin combined to restore all features of the normal curve. Quite possibly a larger number of experiments, employing a somewhat larger dose of insulin and varying doses of sugar would do so. It is readily understood why insulin should restrain the peak effect by making the "sugar vacuum" of the tissues, to use Macleod's term, more effective in drawing sugar away from the blood, and why it should increase the rate of recession. But it requires a nicer balance of factors than our crude methods of injection can supply to restore equilibrium promptly.

DISCUSSION. The findings of previous workers who have employed renal exclusion to test the presence of extra renal effects of phlorhizin are to some extent reconciled by the results here presented. In agreement with Underhill and Csonka it is found that ligation of the renal vessels does give evidence which indicates a lessened tolerance in a phlorhizinized dog. This appears in what we have called the "peak effect" and in the slower deflection to equilibrium (fig. 2). There is some effect of a previous dose of sugar, as Wierzuchowski and Deuel, Wilson and Milhorat have found, and this affects chiefly the rate of recession, particularly in the second phase. When insulin is given both before and after ligation, it may: *a*, simply reduce the effect of ligation, as in figure 5; or *b*, it may completely wipe out the effect, as in figure 6; or *c*, finally, it may reduce

the effect principally in one part of the curve and not in another, as in figure 7. Since some reduction of the effect of stopping the kidney leak occurs in all cases (shown best by contrast of figs. 4 and 5) an attempt will be made to discern the way in which insulin acts. Only two ways seem possible: 1, by imitating or partially accomplishing the effect of ligation before ligation occurs, in which case the tolerance curve just before ligation should run higher than if no insulin were given; or 2, by lowering both curves, the preligation as well as postligation, but since the latter necessarily follows, at least one (fig. 5) and, in these experiments sometimes two, (figs. 6 and 7) previous tolerances, the postligation curve is affected more than the preligation curve, in which case the two curves are brought closer together. The first explanation is equivalent to saying that insulin acts on the kidney to remove, or overcome temporarily, the effects of phlorhizin. The second, that insulin (with sugar) seems to remove in large part the lack of tolerance noted in these animals (dogs 4, 5 and 9) before insulin was administered.

There is independent evidence in support of the former explanation. v. Creveld and v. Dam (23) demonstrated, as they believed, that the permeability of the kidney to perfused glucose was decreased by the addition of insulin to the perfusion fluid. Goldstein and Stephens (24) in this laboratory reported an antagonistic action of insulin to phlorhizin, when insulin was injected slowly into one renal artery of phlorhizinized dogs, and the urine collected separately from the two ureters. In further unpublished experiments of the same kind, performed by others, this antagonism has not been confirmed. Rosenfeld (25) explained the singular resistance of phlorhizinized dogs to large doses of insulin (3-4 U. per kgm.) as due in part to a decrease of renal permeability to glucose. Doses of such magnitude have not been given in the present work. However, in the paper by Doctor Hawley (26) from this laboratory describing the effects of insulin on the D:N ratio and respiratory metabolism of the phlorhizinized dog, in which the doses were comparable to those employed in this work, there is evidence of diminished elimination of sugar. For example, with dog 3 (chart 2, p. 190) even when there was very little effect on combustion, after phlorhizin and insulin, there was obviously a fall in urinary sugar together with a decrease in urinary N to give a practically constant D:N ratio.

The expected effect of a diminished excretion showing in some part of the tolerance curve as the result of insulin alone was not realized in our own experiments possibly because the dose of insulin was not large enough, or, more probably, because the affect on utilization (glycogen formation, combustion) was so much greater than the effect on the kidney. At all events, while it seems to require something more than ligation of the renal vessels and insulin with glucose to restore all features of the normal toler-

ance curve (see p. 180) we have not been able to demonstrate an effect on the kidney.

Kempner (27) has shown that the tolerance curves of phlorhizinized dogs last longer than in normal dogs and he believes that insulin in these dogs does not prevent the hyperglycemia following glucose administration *per os*, although it does prevent in normal dogs.

On the view of Hagedorn (28) that glucose is stored in peripheral tissues during alimentary hyperglycemia, it might be inferred that phlorhizin interferes with this peripheral storage, as well as with liver storage, and that insulin does not at once overcome this interference.

The experiments here presented afford confirmation of Kempner's result with reference to the duration of (intravenous) hyperglycemia, but not with reference to the effect of insulin, unless we count hyperglycemia from the phlorhizin blood sugar level. Further, the observations of Cori (29) and of Nash (30) prove that insulin induces rapid formation of glycogen, whatever may have been the state of depression of this function by phlorhizin (31). The slower recession of the tolerance curve to preinjection level, even when insulin is used, as shown in several experiments of this series, proves, we believe, that phlorhizin has impaired beyond the power of insulin and sugar easily to restore, some mechanism concerned in the removal of sugar from the blood. Since this does not seem to be the power of combustion, what can it be other than permeability of the tissues to sugar similar to the effect in the kidney?

SUMMARY

1. The question of the influence of phlorhizin on the utilization of glucose has been tested by means of the intravenous glucose tolerance, with particular reference to the effects of renal vessel ligation and the effect of insulin injected with the glucose both before and after renal vessel ligation.

2. Two criteria are used to determine the amount of tolerance. With equivalent doses of glucose per unit of weight and the same rate of infusion, tolerance may be judged by a , the peak effect, i.e., height reached by the blood sugar above preinjection level, and b , by the rate of recession, i.e., time to reach some given level, or time to reach equilibrium (constant level), from the peak of the curve.

3. The glucose tolerance curve in the phlorhizinized dog differs from that in the normal animal in two respects; a lower peak effect, and a slower rate of recession.

4. The recession curve is diphasic, being much more rapid the first 20 to 30 minutes. In the normal animal there is a much more abrupt turn to the equilibrium level than in the phlorhizinized animal.

5. A first tolerance affects a second both in the normal and the phlorhizinized animal, probably by inducing the production of insulin. When

insulin is given with the glucose in the normal animal, the two curves may be identical in the later phases.

6. Ligation of the renal vessels in the normal animal does not alter the absolute level of the peak but does raise the "peak effect" as here defined, because the previous tolerance has lowered the starting point. It does not of itself affect the rate of recession.

7. Ligation in the phlorhizinized animal raises the entire tolerance curve (in 3 out of 4 trials) but raises it less when insulin is given with glucose in both tests than when insulin is not given.

8. When insulin is given (with glucose) both before and after ligation, the effect of ligation itself largely or entirely disappears. The significance of this is discussed.

9. If there was any effect on the kidney to antagonize the effect of insulin, it was obscured by the greater effect on utilization.

10. The tolerance curve shows a slower recession to preinjection level, even when insulin is used, and it is believed that phlorhizin has impaired, beyond the power of insulin and sugar easily to restore, some mechanism concerned in the removal of sugar from the blood.

BIBLIOGRAPHY

- (1) NASH, T. P., JR. AND S. R. BENEDICT. *Journ. Biol. Chem.*, 1923, *lv*, 757.
- (2) GAEBLER, O. H. AND J. R. MURLIN. *Journ. Biol. Chem.*, 1925, *lxvi*, 731.
- (3) WIERZUCHOWSKI, M. *Journ. Biol. Chem.*, 1926, *lxxviii*, 385.
- (4) DEUEL, H. J., JR., H. E. C. WILSON AND A. T. MILHORAT. *Journ. Biol. Chem.*, 1927, *lxxiv*, 265.
- (5) MINKOWSKI, O. *Arch. f. exp. Path. u. Pharm.*, 1892, *xxxi*, 85.
- (6) UNDERHILL, F. P. *Journ. Biol. Chem.*, 1912-1913, *xiii*, 15.
- (7) PEARCE, R. G. *This Journal*, 1916, *xl*, 418.
- (8) CSONKA, F. A. *Journ. Biol. Chem.*, 1916, *xxvi*, 93.
- (9) DEUEL, H. J., JR. *Journ. Biol. Chem.*, 1930, *lxxxix*, 77.
- (10) DEUEL, H. J., JR. AND M. GULICK. *Journ. Biol. Chem.*, 1930, *lxxxix*, 93.
- (11) NASH, T. P. *Journ. Biol. Chem.*, 1929, *lxxxiii*, 139.
- (12) GNOINSKI, H. *Compt. rend. soc. biol.*, 1927, *xvii*, 942.
- (13) GNOINSKI, H. *Compt. rend. soc. biol.*, 1928, *xviii*, 785.
- (14) HINES, H. M., J. D. BOYD AND C. E. LEESE. *This Journal*, 1926, *lxxvi*, 293.
- (15) WIERZUCHOWSKI, M. AND H. GADOWSKA. *Biochem. Zeitschr.*, 1917, *cxc*, 398.
- (16) OLMSTED, J. M. D. AND G. GIRAGOSSINTZ. *Proc. Soc. Exp. Biol. Med.*, 1929, *xxvii*, 103.
- (17) COLLENS, W. S. AND J. R. MURLIN. *Proc. Soc. Exp. Biol. and Med.*, 1929, *xxvi*, 485.
- (18) JORGENSEN, S. *Acta med. scand.*, 1926, *lxv*, 116.
- (19) ROSENFELD, G. *Biochem. Zeitschr.*, 1930, *cxxii*, 457.
- (20) DU VIGNEAUD, V. AND W. G. KARR. *Journ. Biol. Chem.*, 1925, *lxvi*, 281.
- (21) LENNOX, W. G. *Journ. Biol. Chem.*, 1927, *lxxiii*, 237.
- (22) FOSTER, G. L. *Journ. Biol. Chem.*, 1923, *lv*, 303.
- (23) VAN CREVELD, S. AND E. VAN DAM. *Arch. neerland physiol.*, 1925, *x*, 323.
- (24) GOLDSTEIN, J. AND D. J. STEPHENS. *Proc. Amer. Physiol. Soc.*, *This Journal*, 1927, *lxxx*, 480.

- (25) ROSENFELD, G. Arch. f. exp. Path. u. Pharm., 1930, clvii, 149.
- (26) HAWLEY, E. E. This Journal, 1932, ci, 185.
- (27) KEMPNER, W. Arch. exp. Path. u. Pharm., 1927, cxxii, 1.
- (28) HAGEDORN, H. C. Physiology papers dedicated to August Krogh, 1926, 80.
- (29) CORI, C. F. Journ. Pharm. Exp. Therap., 1924, xxiii, 99.
- (30) NASH, T. P., JR. Journ. Biol. Chem., 1926, lxvi, 869.
- (31) SCHWARZ, C. AND H. SASSLER. Biochem. Zeitschr., 1928, cxcviii, 250.

STUDIES ON THE POSSIBILITY OF GLUCONEOGENESIS FROM FAT

I. RESPONSE OF THE PHLORIDZINIZED DOG TO INSULIN¹

ESTELLE E. HAWLEY

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

Proof that carbohydrate can be converted into fat in the animal body is not wanting. Rapport (1930) in his excellent review of "The Interconversion of the Major Foodstuffs," cites the experiments of note. It is the reversibility of that reaction, which is at the present time one of the most interesting of physiological questions. The general chemical law of the reversibility of reactions, and the fact that it seems logical that the animal body should be able to utilize its fat stores, which have been built up from carbohydrate, in the formation of new carbohydrate when it is needed, has led many investigators to seek convincing proof of this conversion. Plants apparently convert their fat stores into carbohydrate during the process of germination (Leathes and Raper, 1925). Lower animals, as exemplified by the silk worm (Couveur, 1895) also appear to build up carbohydrate at the expense of fat stores. Hence, it would seem that if such a change does take place in any form of life, it would at least be possible in higher forms, even though it may not be a necessity.

The question has been studied by means of experiments determining the fuel used for muscular work both in isolated muscle and experiments on the subject as a whole, by perfusion experiments, hibernating animals, adrenalin experiments and with diet work.

Depancreatized and phloridzinized dogs have been favorite experimental animals. Nash (1927), Lusk (1928) and Macleod (1926, 1928) review the subject extensively. High D:N ratios are held by many workers to indicate sugar production from a source other than carbohydrate.

The differences in the physical characteristics of experimental animals, it would seem, are not clearly enough recognized by readers in evaluating results of experiments where contradictory evidence is obtained. One

¹ Taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, June, 1931.

finds each investigator employing, for the most part, a somewhat different technic and the results are not always truly comparable.

The variability of the results in human diabetes is great even when conditions are strictly controlled, for it is seldom, if ever, that the pancreas becomes so far destroyed that there is no internal secretion of insulin (Macleod, 1926). Therefore, there may be periods during which carbohydrate is stored and others, when it is being broken down. There is evidence that insulin production varies from time to time in the normal and this is probably the case in the experimental animal.

Geelmuyden (1923) notes the variability in nitrogen output. Great nitrogen retention apparently occurs at times. This point is also discussed by Benedict and Joslin (1911). There is always the difference in rate of excretion of the nitrogen and dextrose, a point which in short period determinations may make a considerable difference.

The question is so controversial and the evidence on each side so incomplete, that it would appear that a crucial experiment must soon be devised which would throw the weight of evidence one way or the other.

Wertheimer (1926d) reports experiments which he says "very nearly, if not entirely, demonstrate the change of fat into carbohydrate." This paper is the fourth in a series on "Stoffwechselregulationen." Briefly, his conception is that insulin may greatly accelerate the change from fat to carbohydrate. This transformation should be a normal process which would take place very slowly. If one were to use, for example, an animal whose liver contains large quantities of fat like a phloridzinized dog, one should, through insulin injections, be able to convert that fat into carbohydrate.

Dogs were the experimental animals chosen by Wertheimer. If his theory were correct, the reaction of an animal whose liver contained large quantities of fat and only traces of carbohydrate, should be very different from that of an animal containing a liver which, through fasting, had been depleted of its glycogen stores. If fat could be transformed into carbohydrate, the sensitivity to insulin should be less.

In Wertheimer's experiments the dogs whose livers contained large quantities of fat due to phloridzin were more resistant to insulin, their blood sugar returned more quickly to the original level and the recovery from insulin shock was much more rapid than in the control dogs not protected by increased fat stores.

EXPERIMENTAL. Since there is no record of D:N or respiratory work in Wertheimer's article, the plan was to include these determinations in a repetition of the experiment. Another modification was to use the same dog for both control and phloridzin experiments.

The dogs were carefully selected, the qualifications being suitable size for the respiration chamber, dogs which could be easily trained, whose veins

were comparatively accessible, and dogs which would eat the diet with enjoyment, in order that assurance might be had that the food would be eaten even when the animals would be expected to lose their appetites. No preference was given to any particular breed of dog. Female dogs were used in all except the first of the seven experiments.

A preliminary tryout was made before the experiment was started in order to test the qualifications of the dog. In spite of this, three dogs did not complete the experiment. The work here reported is on three. The fourth was so sensitive to insulin that no data were obtained in that period.

The plan was to use the dog first as a control and then to phloridzinize and repeat the experiment. It was thought that if this were done, individual variations might be more adequately ruled out. For this reason the diet was carefully planned in order to maintain as nearly as possible the good state of nutrition of the animal. The diet suggested by Cowgill (1923) was adjusted to the dogs, and fed at the end of each day.

With the exception of dog 2, the diet was continued during the phloridzin period.

PROCEDURE. The method of approach in the experiments here reported may perhaps be best explained by including a typical outline of a day's work.

8:00 a.m. Dog weighed and catheterized.

Blood sample taken.

If normal control, dog placed in respiration chamber for basal determinations.

Preliminary period of 20 minutes, followed usually by two basal periods.

10:00 a.m. Dog catheterized, blood taken and insulin injected subcutaneously.

Dog returned to chamber.

Preliminary 20 minutes.

(Periods so timed that each ended at even hours after insulin injection.)

2:00 p.m. Dog removed, usually at end of 4 hours' confinement, though in some cases 6 successive periods were run. Four-hour blood sample obtained.

4:00 p.m. Six-hour urine and blood.

On the following day the blood samples were taken every 2 hours; urine, basal and 6-hour; and if the dog was removed at 4 hours on the previous day, the 5- and 6-hour R.Q.s were obtained on this day.

In the phloridzin experiments the dog was given one gram of the drug in 10 cc. olive oil, in two fractions, 5 cc. in each side of the body at 8:00 a.m. The insulin was given after the basal period, approximately 2 hours after phloridzin and never in the same site as phloridzin.

METHODS. The chemical determinations were as follows: Dextrose in the urine, Benedict's method (1911), nitrogen by the macro-Kjeldahl, blood sugar according to Folin-Wu (1920) and blood fat by Bloor's method (1926).

The respiratory experiments were carried out on the Benedict Universal Respira-

tion Machine described by Hawley and Murlin (1925). The only change was the addition of 150 cc. concentrated NaOH to each 2-quart jar of soda lime. After thoroughly mixing, the soda lime was allowed to stand over night. More efficient CO₂ absorption resulted.

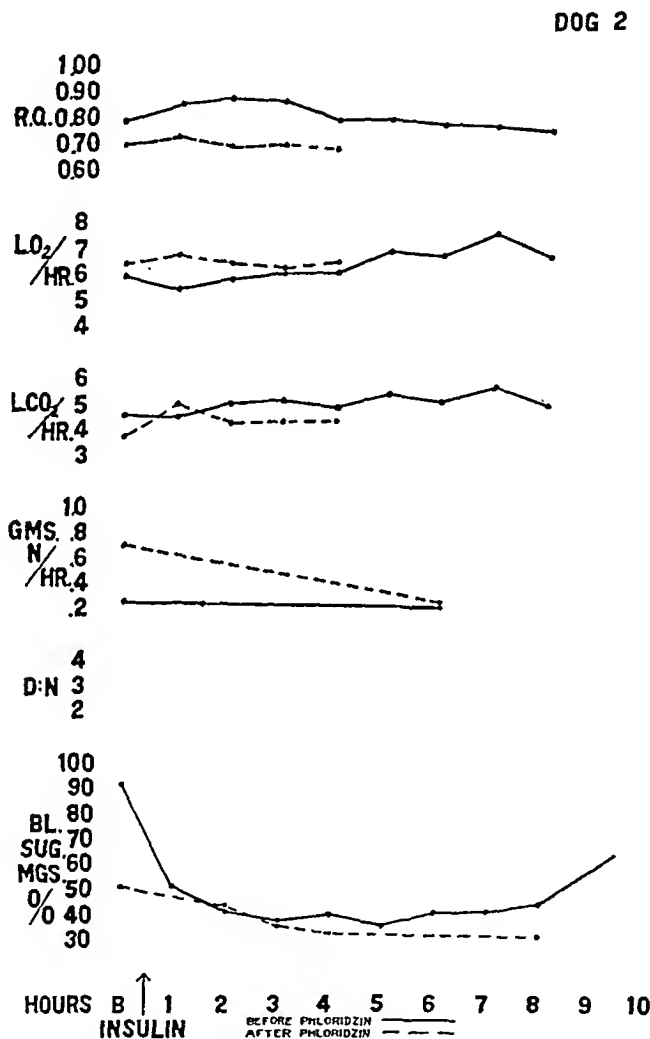


Chart 1

RESULTS. Dog 2, (chart 1) a very fat spaniel, certainly was unable to convert her fat stores into sugar, even in an hour of great need. She finally died in diabetic coma, unable to mobilize enough sugar to burn up her acetone bodies. She should have been ideally equipped to manufacture glucose both from her own considerable fat stores and her fatty liver.

The basal levels in all respects were typical, as was the response to insulin. In general, one would expect a higher R.Q. following insulin than 0.86.

The reaction was greater in the first series, but due to the restlessness resulting from the insulin dosage, a smaller dose was used in the later work.

The results after phloridzinizing are interesting but not in accord with those of Wertheimer. The dog was much more sensitive to insulin after phloridzin than before. Starting with a typical diabetic basal R.Q., there was no marked change in the level. If there had been conversion into sugar, there would have been a greater O_2 consumption and a depression of the quotient. There is a slight increase in O_2 the first hour after insulin, but it goes hand in hand with a greater CO_2 increase with a resulting slight rise in R.Q.

The D:N did not exceed the Lusk ratio (Stiles and Lusk, 1903) for the basal period. No determinations for the insulin period were obtained because sugar was necessary in every experiment to revive the dog.

The nitrogen excretion was more than doubled after phloridzin administration. This was to be expected. A 3 to 5 times increase in nitrogen excretion is reported by Lusk (1915). Wertheimer, however, in the only reference which he makes to protein metabolism, says "it was unchanged," which indicates that the dog could not have been completely phloridzinized.

Macleod (1928) and his pupils think that the D:N is a far less constant figure than one is generally led to believe. The work in his laboratory (Chaikoff, et al., 1925) purports to show that the level is determined by the nutritional state of the animal. A higher level was obtained in a fat dog (exceeding 3.65) than in a very thin dog when the average was nearer 2.80:1. Dog 2, being a very fat dog should, according to Chaikoff's finding, have had a higher D:N or, in other words, have been forming sugar from her fat stores. This idea of the fat stores serving as glucose precursors is nullified to a large extent by the observations of Wertheimer himself (1928) and others (Scoz, 1930), showing that glycogen is found in considerable quantity in the adipose tissue. Moreover, a dog whose nutritional state was such that excessive fat was stored, also must have had large glycogen stores in liver and muscle as well. Such an animal should be depleted by adrenalin until the D:N is constant; otherwise depletion may continue and thereby elevate the D:N above the protein level.

The blood sugar curve before phloridzinization, with insulin alone, rose more slowly than one would ordinarily expect, but it did rise sharply after 8 hours (chart 1). There was abundance of body fat which could have been converted into sugar. The curve following phloridzin started at a lower level, as it should, fell lower, and at eight hours was at its lowest point. It would have been interesting to follow it longer, but sugar administration interfered. It seemed wiser to save the dog than to continue the curve. This blood sugar curve is in sharp contrast with Wertheimer's findings.

The phloridzin experiment would have been continued for a longer time

if the susceptibility to insulin had not been so marked. Each insulin day was interrupted by the giving of sugar to abort the convulsions. In spite of this, the dog died very unexpectedly in a typical diabetic coma. Adrenalin was administered which, according to Wertheimer's theory, should have called out sugar, but evidently none was mobilized, and death

DOG 3

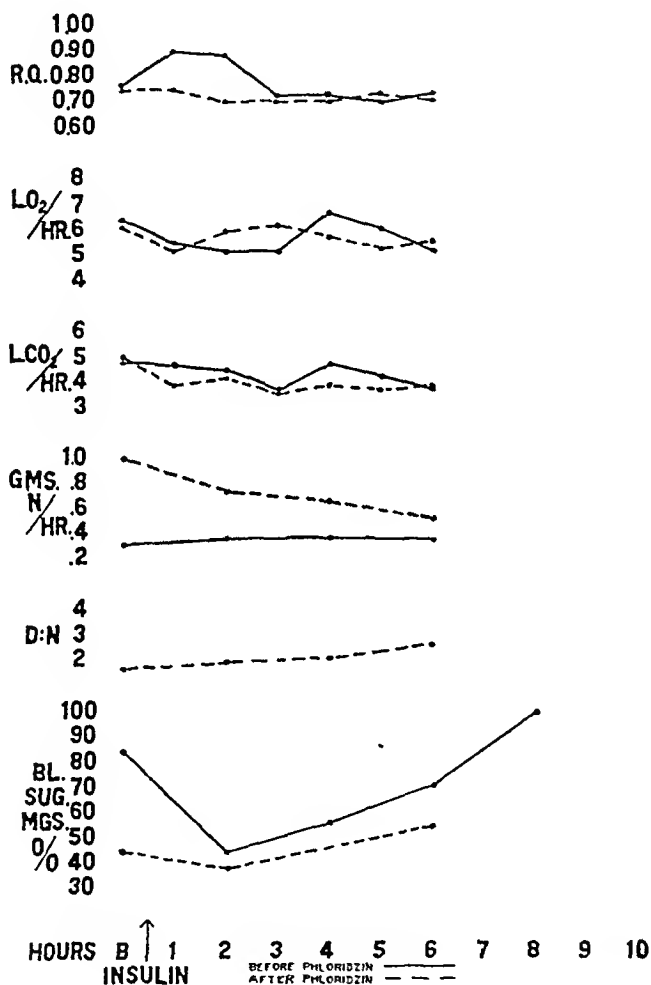


Chart 2

resulted. For several days before her death, this dog had been jaundiced. Bile appeared in the urine and she had the general appearance of a "sick" dog. Meat was offered but was refused even when it was placed in the mouth.

On autopsy much fatty tissue was found and a distinctly yellow liver, indicating large fat deposits.

There was no evidence of any abnormal condition which might have been the cause of death, other than the very evident acidosis. No signs of abscess or infection at the site of any injections were found.

Glycogen determinations on fat of the omentum, muscle and liver were made. A trace was found in the muscles but the liver and fat were nega-

DOG 5

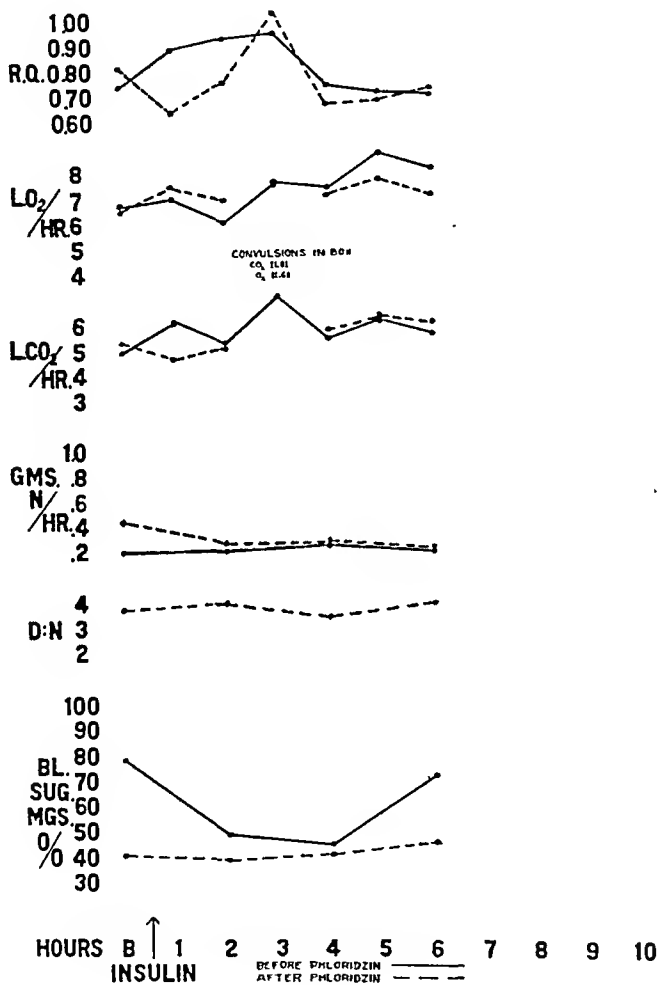


Chart 3

tive, both with a Folin-Wu and a Benedict reading of the final sugar. There may have been a loss due to time elapsing between the death of the animal and placing the samples in NaOH. Since the death of the animal was unexpected, preparations were not made for the analyses. The general opinion has prevailed that glycogen is rapidly broken down after death, but Kira (1922) has found that the loss is not at all proportional to

time and that not more than 50 per cent is lost from the rat liver in 3 to 5 hours even when the initial glycogen is high (4-7 per cent).

The fat determination showed 56 per cent fatty acid in the liver and 73 per cent in the fatty tissue. Evidently the fat stores had not been depleted to any large extent for conversion into glycogen or sugar.

This dog then very definitely did not bear out Wertheimer's theory.

Thinking that starvation during the phloridzin period might possibly have decreased the resistance of dog 2 to insulin, it was decided to repeat the experiment and continue the feeding throughout. The difficulty which is experienced by some in feeding phloridzinized dogs was not met with in any of the dogs here used.

In dog 3 (chart 2) the initial response to insulin was slightly greater, as evidenced by the higher quotient. The blood sugar rose rapidly after the low point at 2 hours and at 8 was 15 mgm. above the basal. This dog, in contrast with dog 2, was very thin and yet, throughout the experiment was more resistant to insulin. Again there was no R.Q. change after phloridzin which could indicate conversion. The D:N at no time exceeded the normal ratio. The blood sugar did rise, however, as it had in the control insulin period though more slowly.

The average curves for dog 5 (chart 3) vary somewhat. The R.Q. was not so different since there was a compensatory fluctuation in both the CO_2 and O_2 .

The low point in the blood sugar was at 4 hours, though at 2 hours there had already been a very decided drop.

After phloridzin, the third hour in the respiration chamber, the dog went into convulsions and finally lay prostrated on the bottom of the chamber. It was decided to continue the experiment in spite of this. The R.Q. as a result rose to 1.08 and the O_2 and CO_2 were approximately doubled.

The D:N in this dog exceeded the normal ratio for a time but this was due rather to low nitrogen than to a high dextrose. This ordinarily is interpreted to mean that extra carbohydrate is coming from a source other than protein; but if it did, the animal, in this instance, did not use it as a means of resisting insulin. In the next two attempts to follow the insulin reaction, convulsions developed which were so severe that sugar had to be given and the experiment discontinued.

In conclusion one may say that from the evidence here presented there is no conversion of fat to carbohydrate under the influence of insulin. Even when there was a very great need of carbohydrate to save the life of the animal, and when there were ample stores of fat or ample food fat available to meet this need, there was no gluconeogenesis from fat.

Appreciation to Dr. John R. Murlin is gratefully acknowledged for his suggestions throughout this research.

BIBLIOGRAPHY

- BENEDICT, F. G. AND E. JOSLIN. 1910. Metabolism in diabetes mellitus. Carnegie Publ. no. 136.
- BLOOR, W. R. 1926. Journ. Biol. Chem., lxxvii, 53.
- CHAIKOFF, I. L., J. J. R. MACLEOD, J. MARKOWITZ AND W. W. SIMPSON. 1925. This Journal, lxxiv, 36.
- COUVEUR, M. E. 1895. Compt. Rend. Soc. Biol., xlvii, 796.
- GEELMUYDEN, H. C. 1923. Ergebn. d. Physiol., xxii, 51.
- HAWLEY, E. E. AND J. R. MURLIN. 1925. This Journal, lxxii, 264.
- LEATHES, J. B. AND H. S. RAPER. 1925. The fats. London.
- LUSK, G. 1928. Science of nutrition. 4th ed., Philadelphia.
1915. Arch. Int. Med., xv, 939.
- MACLEOD, J. J. R. 1926. Carbohydrate metabolism and insulin. New York.
1928. The fuel of life. Princeton, N. J.
- KIRA, G. 1922. Mitth. a. d. med. Fak. d. Kais. Univ. du Tokyo, xxx, 65.
- NASH, T. P. 1927. Physiol. Rev., vii, 385.
- RAPPORT, D. AND E. P. RALLI. 1928. This Journal, lxxxiii, 430.
- SCOZ, G. 1930. Chem. Abstracts, xxiv, 5826.
- STILES, P. G. AND G. LUSK. 1903-4. This Journal, x, 67.
- WERTHEIMER, E. 1926a, b, c, d. Pflüger's Arch., ccxiii, 262-320.
1928. Pflüger's Arch., ccxix, 190.

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

II. FURTHER STUDIES ON RESPIRATORY METABOLISM OF ANESTHETIZED DOGS¹

E. S. NASSET

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

In an earlier paper (Nasset, Bishop and Warren, 1931) we reported the changes in respiratory metabolism of eight anesthetized dogs treated with high frequency electric current. The experiments reported here include some data obtained using ten times the frequency employed in the earlier work, as well as some determinations made by another method but using the lower frequency.

It was pointed out in the first paper of this series that in certain instances it seemed that the rate of oxygen consumption was dependent upon the passage of current more than upon body temperature and the rate of respiration. The demonstration of such a "specific effect" of high frequency current would be of very great interest. Plaut and Wilbrand (1922) noticed an excessive oxygen consumption as a result of exposure to a bank of high power electric lights. Bazett suggested (1927) that the alkalinity of the blood caused from the overventilation and consequent loss of carbon dioxide might influence oxygen uptake in some way yet unknown. In acute experiments, such as reported here, it must be borne in mind that the energy required for the respiratory muscles is very great. In experiments on three human subjects, Liljestrand (1928) found that under basal conditions the energy required for respiratory movements was 1 to 3 per cent of the total metabolism. He demonstrated very great increases in oxygen consumption when the work of the respiratory muscles was increased either by rebreathing carbon dioxide or by voluntary forced breathing. In the latter instance he was able to increase the total metabolism 4.4 per cent for every "extra" liter of pulmonary ventilation per minute. If these data can be applied in the interpretation of our results on anesthetized dogs, it is quite likely that all of the oxygen consumed can be accounted for on the basis of the heat effect and the muscular work entailed in the excessive pulmonary ventilation.

¹ Taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, June, 1931.

METHODS. In the experiments in which the Benedict Universal metabolism apparatus was used, the technique was essentially the same as described in our first paper. Beginning with experiment 34 the dogs were fasted at least 40 hours. The metabolism was determined by the collection and analysis of expired air. A very accurately counterpoised gasometer of the Tissot type (150 liter capacity) was constructed for these experiments. The volume of expired air can be measured with an error of less than 0.5 per cent. The gas analyzer, of a modified Haldane type, was also constructed especially for use in the work herein described; the burette has a capacity of 20 cc., the lower 6 cc. portion being calibrated directly in hundredths. The volume of gas in the burette can be estimated with an accuracy of plus or minus 0.001 cc., corresponding to 0.005 per cent of the total volume. In the carbon dioxide and oxygen determinations, this error may be doubled due to manipulation of solution levels and the like, so that the probable limit of accuracy for this determination is plus or minus 0.01 per cent. A glass cannula connected the dog's trachea with a pair of Bailey flutter valves. Inspired air was taken from outdoors. A pneumograph recorded the respirations on a smoked paper by means of a tambour which also controlled the circuit of an electrical counting device. In this manner we were enabled to determine the total number of respirations in any given interval of time and to estimate the tidal volumes.

RESULTS. Illustrative results are given in tables 1 and 2. There is no apparent difference between the response previously obtained at 10^6 cycles and the response to the current of higher frequency (expts. 14, 15, 16). In experiment 14 the metabolism attained a value of 176 per cent above the basal, showing an average increase of 33 per cent per degree rise in body temperature. Experiment 15 extended over a temperature range 0.3° greater than the previous one, but the metabolism rose only 90 per cent above basal. In experiment 16, as in a number of others, an increase in metabolism was noted during the period immediately following the shutting off of the current. With one exception (expt. 16) this was accompanied by a greater ventilation or a higher body temperature, or by a combination of both (expts. 16, 17, 18). Experiments 17, 18, 19 and 20 were carried out in the same manner as those described in the first paper. The increase in energy output in these experiments ranged from 24 per cent per degree rise in body temperature in experiment 20, to 45 per cent per degree in experiment 19 (313 per cent above basal). It should be noted that the current in some of these experiments was switched on and off at intervals in order to detect, in this manner if possible, any "specific effect" which the current might exhibit.

Table 2 represents some of the experiments done by the method of collection and analysis of expired air. The anesthetic used was sodium amytal. These experiments give us some information in regard to the relative increase in pulmonary ventilation, thus making possible a more accurate

TABLE 1

Respiratory metabolism of anesthetized dogs treated with high frequency electric current

EXPERIMENT NUMBER AND DATE	AVERAGE BODY TEMPERA- TURE	METABOLISM PERIOD	TIME END OF PERIOD	TOTAL CALORIES PER HOUR AT OBSERVED R.Q.	OBSERVED R.Q.	RESPIRA- TIONS PER MINUTE
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(10⁷ Cycles. No contact electrodes)
Benedict universal metabolism apparatus

	°C.					
14(9-25-30)	35.6	1	2:31	23.4	0.88	5
Current on	35.9	2	2:55	26.5	1.08	8
Current on	38.8	3	3:21	41.1	1.38	90
Current on	41.0	4	3:49	64.5	0.96	—
Wt. 15.6 kgm.			4:07	Death		
15(10-23-30)	36.7	1	2:39	20.5	0.83	—
Wt. 11.9 kgm.	36.3	2	3:11	20.5	0.81	—
Current on	37.5	3	3:42	23.7	0.88	—
Current on	41.2	4	4:19	39.0	0.97	—
Current on	44.1	5	4:38	37.0	0.93	—
			4:40	Death		
16(1-27-31)	36.6	1	11:40	29.9	0.91	5
Wt. 24.3 kgm.	36.4	2	12:07	28.3	0.90	—
Current on	39.2	3	12:41	37.2	1.23	38
Current on	41.3	4	1:09	61.2	1.11	174
Current off	40.9	5	1:44	69.6	0.94	—
Current off	40.5	6	2:19	56.0	0.85	182
Current off	39.8	7	3:04	54.0	0.76	140
Current on	41.5	8	3:30	47.1	1.10	103
Current on	45.8	9	3:59	59.7	0.96	160
			4:04	Death		

(10⁶ Cycles. Arm-leg electrodes)

19(2-6-31)	36.5	1	11:50	24.4	0.78	10
Wt. 21.2 kgm.	36.1	2	12:26	23.6	0.84	12
Current on	35.8	3	2:27	23.5	0.75	10
Current on	36.3	4	3:04	27.0	0.87	11
Current on	37.8	5	3:41	29.8	0.89	12
Current on	38.7	6	4:17	34.4	0.89	11
Current on	40.1	7	4:58	46.5	1.01	190
Current on	40.8	8	5:34	56.3	0.90	200
Current on	43.2	9	6:05	99.1	0.87	150
			6:17	Death		
20(2-9-31)	36.9	1	12:07	22.7	0.76	10
Wt. 20.0 kgm.	36.4	2	12:40	22.2	0.83	11

TABLE 1—*Concluded*

EXPERIMENT NUMBER AND DATE	AVERAGE BODY TEMPERA- TURE	METABOLISM PERIOD	TIME END OF PERIOD	TOTAL CALORIES PER HOUR AT OBSERVED R.Q.	OBSERVED R.Q.	RESPIRA- TION PER MINUTE
(10 ⁶ Cycles. Arm-leg electrodes)— <i>Concluded</i>						
	°C.					
Current on	37.9	3	1:31	26.5	1.07	36
Current on	40.3	4	2:15	51.2	0.86	107
Current on	41.2	5	2:51	55.3	0.76	335
Current off	39.3	6	3:28	46.0	0.78	385
Current off	38.5	7	4:08	29.1	0.71	26
Current on	41.1	8	4:46	33.1	0.84	72
Current on	42.4	9	5:22	49.7	0.85	247
Current on	43.7	10	5:58	60.9	0.83	240
			6:13	Death		

accounting of the heat production of these animals. In addition there are data on a dog with a fever of infectious origin, and on a control animal. The rectal temperatures were recorded at the beginning and at the end of the metabolism period—the length of the periods varied from 10 to 4 minutes, depending upon the rate of ventilation. The greatest increase above basal (117 per cent) was obtained in experiment 34. The increase in energy production per degree rise in rectal temperature varied from 10–20 per cent for the dogs receiving the current. For the animal with the fever, the total increase was only 16 per cent above the value at the beginning of the experiment—9 per cent per degree rise in temperature. Since the effects of amytal on energy metabolism have been thoroughly studied by Deuel, Chambers and Milhorat (1926) it was deemed unnecessary to perform a great number of control experiments. The two controls reported in the previous work and the one reported here, are the only ones which we have completed to date. In each case the result has been essentially the same, i.e., either there is no great change or a tendency toward a decreased heat production.

DISCUSSION. It is obvious that a temperature coefficient (Q_{10}) of metabolic processes of 2.3, such as found by DuBois (1921) for febrile patients, will not account for the enormous increases in heat production noted in the experiments here described. Employing the formula

$$\log Q_{10} = \frac{\log k_1 - \log k_2}{t_1 - t_2} \times 10 \text{ (Bazett, 1927)}$$

to calculate the Q_{10} from DuBois' graph representing the average metabolism at different temperatures, from 37° to 40°, we were unable to obtain a value of 2.3. According to this graph, the average rise in metabolism from

37° to 40° is 34 per cent. Now, if we take 40 Cals. per square meter as a starting point, i.e., at 37°, the value at 40° is 53.6 Cals. Substituting these values in the above equation we obtain $Q_{10} = 2.65$. In a series of chemical reactions, taken from other sources, plotted in the same manner as the

TABLE 2

Respiratory metabolism of anesthetized dogs treated with high frequency electric current

(10⁶ cycles. Thorax-back electrodes. By collection and analysis of expired air)

EXPERIMENT NUMBER AND DATE	RECTAL TEMPERA- TURE	TOTAL CALORIES AT OB- SERVED R.Q.	OB- SERVED R.Q.	RESPI- RATIONS PER MIN- UTE	VENTI- LATION RATE	TIDAL VOL- UME	PER CENT CO ₂ IN EX- PIRED AIR	PER CENT O ₂ IN EX- PIRED AIR	WEIGHT OF DOG
	°C.				liters per minute	cc.			kgm.
35(3-30-31)	37.2-37.0	36.5	0.89	11	4.12	375	3.01	17.66	24.1
Current on	39.2-39.8	43.8	0.83	14	4.94	353	2.85	17.64	
Current on	40.8-41.4	44.7	0.99	56	13.36	248	1.50	19.45	
Current on	41.9-42.2	58.8	0.85	104	21.02	202	0.94	19.89	
Current off	40.8-40.4	48.0	0.81	39	10.74	275	1.43	19.28	
Current off	39.2-38.9	45.3	0.78	21	6.69	319	2.09	18.42	
Current on	40.4-41.2	54.6	0.81	36	10.04	279	1.73	18.92	
Current on	42.8-43.0	72.2	0.82	174	30.41	179	0.78	20.05	
37(4-3-31)	37.6-37.6	24.3	0.84	6	2.56	427	3.11	17.39	18.8
Current on	39.4-39.8	26.2	0.87	17	4.61	271	2.21	18.49	
Current on	40.4-40.4	36.6	0.94	202	27.21	135	0.51	20.43	
Current on	42.4-42.6	45.5	0.94	175	35.39	202	0.49	20.45	
Current on	43.1-43.1	47.8	1.07	170	39.27	231	0.51	20.47	
38(4-6-31)	39.6-39.7	41.0	0.82	12	4.31	351	3.03	17.42	19.0
Dog with in- fective fever	40.1-40.2	41.6	0.78	13	4.43	341	2.86	17.45	
	40.5-40.6	40.8	0.74	14	4.53	324	2.64	17.59	
	40.8-40.9	44.0	0.78	19	6.33	333	2.16	18.33	
	41.4-41.5	46.7	0.77	35	9.11	260	1.59	19.01	
40(4-24-31)	37.9-37.8	29.0	0.82	9	2.75	306	3.35	17.04	21.1
Control	38.2-38.0	28.6	0.73	10	2.61	261	3.18	16.87	
	38.0-38.0	30.9	0.78	9	2.79	310	3.38	16.81	
	38.1-38.1	28.3	0.70	6	2.39	398	3.37	16.47	
	38.0-38.0	29.5	0.77	7	2.64	377	3.43	16.74	

metabolism experiments, DuBois reports Q_{10} values which we cannot duplicate. For instance, in one case the reaction is accelerated 30 per cent with the temperature rising from 37° to 40° and the Q_{10} indicated on the graph is 2.0. To keep the calculation in Cals. let us assume that the velocity of the reaction at 37° is represented by the production of 40 Cals.

per hour. Hence at 40° the velocity will be 52 Cals. per hour. This, according to the equation cited above, will give $Q_{10} = 2.40$. Now if we calculate the value for Q_{10} between 37° and 38° , we obtain a value of 2.59; if we use the interval 37° to 39° , we obtain $Q_{10} = 2.49$. If the straight line used by DuBois to represent the reaction in question be extended, it will at 47° indicate 100 per cent increase in reaction velocity but this does not indicate $Q_{10} = 2.0$ for the values given in the lower temperature range. From these considerations it appears that the data of DuBois give a Q_{10} somewhat higher than 2.3. There is no doubt that these data show a definite and rather uniform relation between the heat production of febrile patients and their body temperature, but we do not believe that $Q_{10} = 2.3$ expresses this relation. Since we get higher values by using the smaller temperature differences than if we use the three degree range, we have chosen to use $Q_{10} = 2.65$ in attempting to account for a portion of the heat production of the animals used in the experiments described in this paper.

The Q_{10} values, calculated from one metabolism period to the next, show very great variations,—a range of 1.1 to 15.5 (expt. 35) being not uncommon. Similar values can be obtained from the data of others using hot air or hot water to elevate the body temperature. The results of McConnell, Yagloglou and Fulton (1924) show that on exposure to hot, humid air the oxygen consumption was in many cases excessively high, giving some Q_{10} values as high as 49.2. Plaut and Willbrand (1922) report an experiment on a human subject from which a $Q_{10} = 13.4$ may be calculated. The experiments of Landis and co-workers (1926) with hot water baths, of Koehler (1923) and of McConnell and Yagloglou (1925) furnish data of the same general type. These facts demonstrate the very striking difference between the heat production of febrile patients and that of experimental subjects whose body temperature is elevated by extrinsic means. Rapid elevations in body temperature nearly always increase the activity of the respiratory system. This is especially true in the dog where nearly all of the heat loss by evaporation of water must occur in the respiratory tract.

The great increase in pulmonary ventilation is probably the factor largely responsible for the high heat production found in our experiments. In the experiments done with the Benedict Universal apparatus, only the rate of respiration can be accurately recorded, whereas with the collection of expired air both the rate of respiration and the volume of expired air can be accurately measured. The data in figure 1 show that the ventilation may be increased fifteen-fold (expt. 37). The low percentage of carbon dioxide in the expired air indicates an extreme "auspumpung." These conditions are very similar to those which obtain in voluntary forced breathing. Liljestrand (1918) was able to demonstrate more than 4 per cent increase in metabolism for every "extra" liter of expired air

during forced breathing experiments. If in our experiments we calculate the heat production to be expected on the basis of $Q_{10} = 2.65$ and add to this the increase expected on the basis of Liljestrand's work on excessive pulmonary ventilation, we obtain in nearly every case a value far in excess of what was actually found. Of course, there are objections to a calculation of this sort. Liljestrand's experiments were performed on normal human subjects and it may be argued that his data are inapplicable to experiments on dogs under anesthesia. On the other hand, there are several aspects of the two investigations which are quite similar, viz.: 1, there is an alkalosis of the blood; 2, the carbon dioxide content of the expired air is very much lowered, and 3, the active *expiratory* movements

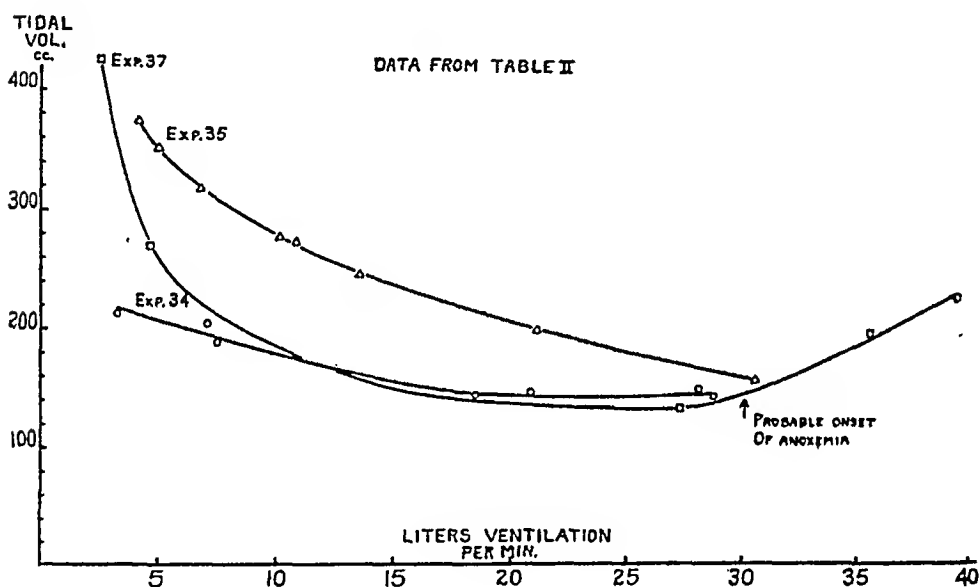


Fig. 1

of Liljestrand's subjects are most certainly duplicated in our experiments on dogs where the respiratory rate attained values of 100 or more per minute.

It is realized that the calculation of the total heat produced in these experiments cannot be absolutely accurate. Almost invariably in the first two or three metabolism periods following the basal there is an elevation of the respiratory quotient. The analyses of expired air and the ventilation rate show that this is due in a large measure to a blowing off of carbon dioxide (see also the third paper in this series). It was shown by blood sugar analyses (Nasset, Bishop and Warren, 1931) that the carbohydrate stores in this type of experiment are drawn upon rather heavily. This fact would also tend to raise the R.Q. That the 40-44 hour fast previous to the

experiments was insufficient to appreciably reduce the carbohydrate stores of the animals, is evidenced by the basal R.Q.s (table 2). Furthermore, the dogs used in these experiments were kept on the stock diet which is rich in carbohydrate. It is believed, therefore, that a calculation of the heat based on the calorific value of oxygen at the observed R.Q. is the best approximation possible under the circumstances.

It was pointed out earlier in this paper that a calculation of the temperature coefficients for heat production in an animal whose body temperature had been rapidly elevated, by hot air or hot water baths, showed the same or greater variation than has been obtained in our experiments with high frequency electric current. Most of the reports of the other work which has been referred to do not contain data from which one may gain information on the relative activity of the respiratory muscles at the elevated body temperatures. The respiratory rate alone does not give the information sought.

Since results similar to ours have been obtained by others without the use of the high frequency current, we cannot say, as yet, that the passage through the body of electric current of the frequencies employed has any peculiar or specific effect on the energy metabolism of anesthetized animals. The decision as to whether there is any such effect depends on a quantitative determination of the energy requirement of breathing at the rapid rates which we have observed. Work on this phase of the problem is in progress and we hope to report these data in a later paper in this series.

SUMMARY AND CONCLUSIONS

The respiratory metabolism of anesthetized dogs treated with high frequency electric current was further studied with the Benedict Universal apparatus and by the collection and analysis of expired air. The maximum observed increase in heat production above basal was 313 per cent. A current of 10^6 cycles per second apparently elicited the same response as a current of 10^7 cycles per second. The pulmonary ventilation was increased up to fifteen-fold with a simultaneous reduction of the carbon dioxide content of expired air to 0.5 per cent. Evidence is presented which suggests that there is no "specific effect" of the high frequency current on the oxygen consumption of the experimental animals.

It is a pleasure to acknowledge the advice and interest of Professor John R. Murlin, the assistance of Messrs. John Karr and H. Harrison, and the technical assistance of Mr. Leo Hofschneider, during the prosecution of this work.

BIBLIOGRAPHY

- BAZETT, H. C. 1927. *Physiol. Rev.*, vii, 531.
DEUEL, H. J., JR., W. H. CHAMBERS AND A. T. MILHORAT. 1926. *Journ. Biol. Chem.*, lxi, 249.

DuBois, E. F. 1921. Journ. Amer. Med. Assoc., lxxvii, 352.

KOEHLER, A. E. 1923. Arch. Int. Med., xxxi, 590.

LANDIS, E. M., W. L. LONG, J. W. DUNN, C. L. JACKSON AND U. MEYER. 1926. This Journal, lxxvi, 35.

LILJESTRAND, G. 1918. Skand. Arch. f. Physiol., xxxv, 199.

McCONNELL, W. J. AND C. P. YAGLOGLOU. 1925. Arch. Int. Med., xxxvi, 382.

McCONNELL, W. J., C. P. YAGLOGLOU AND W. B. FULTON. 1924. Pub. Health Reports, xxxix, 3075.

NASSET, E. S., F. W. BISHOP AND S. L. WARREN. 1931. This Journal, xcvi, 439.

PLAUT, R. AND E. WILBRAND. 1922. Zeitschr. f. Biol., lxxiv, 191.

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

III. THE CARBON DIOXIDE AND OXYGEN CONTENT AND CAPACITY AND THE CONCENTRATION OF BLOOD OF ANESTHETIZED DOGS¹

E. S. NASSET

*From the Department of Vital Economics, University of Rochester,
Rochester, N. Y.*

Received for publication January 26, 1932

A considerable number of observations have been made on the influence of fevers and high environmental temperatures on the concentration of the blood and on the blood gases. Aside from observations made with the intact animal, numerous investigations have been carried out on the physico-chemical behavior of blood at different temperatures *in vitro*. The observations made on experimental animals and human subjects treated with the high frequency electric current are relatively few and it was with the intention of supplementing these data that the experiments described in this paper were carried out. Bischoff and co-workers (1930, 1931a, 1931b) have recently published papers dealing with the blood chemistry of human subjects after treatment with high frequency current. Knudson and Schaible (1931) have reported studies on the blood chemistry of unanesthetized dogs exposed to an ultra-high frequency field. Bourne (1926) made a few observations on the effect of diathermy on the acidosis of ether anesthesia in dogs. Perkins (1931) studied the influence of diathermy treatment upon the blood chemistry of patients with dementia paralytica.

METHODS. The dogs used in these experiments were mongrels weighing from 9 to 27 kgm., obtained from stock. The animals for which hematocrit determinations are reported also served in the metabolism experiments described in the first two papers of this series (Nasset, Bishop, and Warren, 1931) (Nasset, 1932). These dogs were fasted 18 hours. Morphine-sodium amytal anesthesia was used. The hematocrit determinations were obtained incidentally from the centrifuged blood samples used in making the plasma carbon dioxide content estimations reported in the first paper. The samples were centrifuged for 20 minutes at 2300 r.p.m. The centrifuge tubes were graduated in tenths of a cubic centimeter. Five cubic centimeters or more of blood were used and the volumes estimated to the near-

¹ Taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, June, 1931.

est 0.05 cc. The method, therefore, does not have an accuracy greater than plus or minus 1.0 per cent, but because of the gross changes noted and the number of observations made, we believe the data are worth reporting. Beginning with experiment 27 on blood gases, all dogs were fasted at least 40 hours. In experiments 27 to 31 inclusive, sodium amytal anesthesia was used; in the remaining ones morphine-sodium amytal was employed, the amytal being in all cases given intravenously. The samples for blood gas determination were drawn either under paraffin oil or over mercury in tubes similar to those described by Austin, *et al.* (1922). The blood was taken from the saphenous vein, except in a few instances in which it was taken from the external jugular vein. Neutral potassium oxalate was the anticoagulant used. All blood gas estimations were made with the aid of a Van Slyke-Neill (1924) portable manometric apparatus.

Determinations made in duplicate in experiments 29 and 30 show a maximum variation of 0.3 volume per cent for both carbon dioxide and oxygen. Van Slyke and Neill (1924) suggest the determination of the dissolved nitrogen at the end of an analysis, as a check on the completeness of the absorption of oxygen and of the removal of atmospheric air from the reagents. They give 1.2 and 1.4 volumes per cent as the average values for dissolved nitrogen in the blood, depending upon whether the determination is made on blood as drawn, or after saturation with air at 20°C. Our average value for 16 determinations, some made on blood as drawn and some after equilibration with alveolar air at room temperature, is 1.3 volume per cent.

Whole blood was used, care being taken to have the sample well mixed before the analysis was made. For the carbon dioxide and oxygen capacity determinations, about 1.5 cc. of whole blood were placed in a separatory funnel and equilibrated at room temperature with alveolar air for two periods of one minute each. Three determinations on the blood of a neurotic patient are reported. The current used was delivered by two types of apparatus,² one with a frequency of 10^6 cycles per second and the other with a frequency of 10^7 cycles per second. The latter was used only in experiments 14, 15, and 16 (hematocrit). With the latter type of machine the animal was placed in the high frequency field between condenser plates, without any contact electrodes. With the lower frequency apparatus, block tin electrodes were placed on the bare skin, moistened with strong soap suds, of either the legs or the back and thorax. Temperatures were measured by mercurial mouth and rectal thermometers and by needle thermocouples. Complete autopsies were performed on most of the animals.

RESULTS. Illustrative results are given in tables 1 and 2. The rela-

² We are indebted to Dr. S. L. Warren of the Division of Radiology, Department of Medicine, for the loan of the apparatus.

TABLE 1

Hematocrit determinations on anesthetized dogs treated with high frequency electric current

(Illustrative experiments from a total of 11)

EXPERIMENT NUMBER AND DATE	TIME	RESPIRA- TIONS PER MINUTE	BODY TEMPERA- TURE	PER CENT CELLS	REMARKS
			°C.		
9(3-10-30)	4:12	14	35.6	44	Morphine—Amytal 2:15
Current on	4:53	21	36.3	46	Last sample taken from the
Current on	5:21	135	39.4	48	heart
Current on	5:55	250	42.0	52	
Current on	6:44	250	43.9	59	
Current on	6:50	250	43.9	71	
12(7-24-30)	12:08	5	34.1	46	Morphine—Amytal 9:40
Control	1:30	5	34.0	44	
	3:30	6	34.4	43	
13(9-5-30)	11:55	12	34.8	57	Morphine—Amytal 10:20
Control	1:00	11	34.9	56	
	2:00	12	34.2	53	
	3:00	9	32.8	49	
16(1-27-31)	11:45	5	36.6	35	Morphine—Amytal
	12:15		36.2	33	
Current on	12:45	38	39.7	38	10 ⁷ cycles per second
Current off	1:20	180	41.2	43	
Current off	1:52	185	40.7	57	
Current off	2:33	185	40.1	41	
Current on	3:07	70	39.8	63	
Current on	3:58	150	45.6	47	
18(2-4-31)	11:07	30	38.6	42	Morphine—Amytal 9:40
	11:51	28	38.2	38	
Current on	12:47	200	41.9	37	
Current off	1:25	204	41.2	51	Jugular sample
Current off	2:07	165	40.4	39	Jugular sample
Current off	2:48	40	39.8	48	Jugular sample
Current on	3:29	28	39.5	47	Jugular sample
Current on	4:11	200	46.0	50	Jugular sample
19(2-6-31)	11:55		36.3	33	Morphine—Amytal 10:25
	12:30		36.0	33	
Current on	2:32		36.2	36	
Current on	3:09		36.8	37	
Current on	3:45		38.3	40	
Current on	4:26		39.3	33	
Current on	5:02		40.3	44	
Current on	5:38		40.9	49	
Current on	6:09		46.5	57	

TABLE 2

Blood gas determinations on anesthetized dogs and one patient treated with high frequency current

(Illustrative experiments from a total of 11)

EXPERIMENT NUMBER AND DATE	TIME	BODY TEMPERATURE °C.	WHOLE BLOOD						CURRENT	REMARKS
			CO ₂ content vols. per cent	CO ₂ capacity vols. per cent	O ₂ content vols. per cent	O ₂ capacity vols. per cent	Hemoglobin sat- uration	Respirations per minute		
24(2-18-31) Patient F. P.	10:15	36.9		57.9		18.8		28	On	Liquids taken during treatment
	1:15	39.8		55.5		22.6		36	On	
	3:20	40.4		53.4		22.2		40	On	
25(2-20-31)	10:40		40.9	52.9	21.3	25.0	85		On 11:54	Amytal 10:45
	11:50	38.6	50.7	52.9	14.8	19.9	74	11	On	
	1:14	39.7	48.7	53.0	18.2	19.8	92	10	On	
	2:45	40.5	47.9	53.9	17.9	18.3	98	70	On	
	3:55	41.8						150	Off 3:17	
	4:47	43.2	39.2	49.3	14.9	22.1	67	42	On 4:37	
27(2-27-31)	9:30		39.2	47.1	18.2	25.1	73		On 10:45	Amytal 9:40
	10:40	38.2	40.9	51.9	20.3			20	On	
	12:10	40.0	43.0	54.9	17.1	19.4	88	30	On	
	2:25	41.0	40.1	52.0	18.2	21.8	84	157	On	
	3:30	41.9	33.4		21.3			217	On	
	4:43	43.1	29.5	44.2	20.8	31.6	66	110	On	
29(3-6-31)	8:54		36.7	45.3	24.3	29.2	83			Amytal 9:00
	11:28	37.9	46.6	53.0	21.6	24.0	90	13	On 11:33	
	12:13	39.5	46.4	53.4	19.8	23.7	84	19	On	
	12:38	41.0	44.2		19.7			53	On	
	1:18	42.8		53.4		23.5		65	On	
	1:55	44.0	39.0	45.7	15.8	25.7	62	110	On	
30(3-9-31)	9:08		37.9	42.1	19.3	23.2	83		Control	Amytal 9:20
	11:20	37.8	42.6	49.9	19.1	22.1	86	9		
	12:20	37.9	42.5	46.8	17.6	22.3	79	9		
	1:52	38.3	41.9	46.3	18.1	20.8	87	10		
	2:55	38.6		46.1		20.2		14		
	3:45	39.0	41.4	46.2	18.7	22.6	83	14		
31(3-11-31)	10:10		42.9	48.5	18.1	21.7	83			Amytal 10:20
	11:25	37.3	53.4	54.3	10.8	15.4	70	8	On 11:35	
	12:25	39.8	49.5	55.5	13.6	18.9	72	35	On	All samples except #1 from ext. jugular vein
	12:45	41.0	46.7		14.1			104	On	
	1:25	42.3	40.4	54.6	13.7	19.7	70	195	On	
	2:00	43.3	37.0	49.3	10.9	23.3	47	126	On	

tive cell and plasma volumes show almost incredible changes. For example, in experiment 9 the cell volume was increased a maximum of 61 per cent and in experiment 16, 85 per cent (table 1). In the control experiments there were decreases in cell volume of 7 and 14 per cent respectively. In the 11 dogs studied in this series, the cell volumes varied from 30 to 57 per cent of the whole blood in the initial sample, with an average of 42 per cent. The percentage increase in cell volume per degree C. rise in body temperature varied from 2.1 to 10.1 per cent, with a mean value of 5.3 per cent. At autopsy the spleens of the animals were often found to be twice normal size and literally packed with very thick blood. Cook and Rose (1930) observed that the spleen of the cat became very much enlarged following amytal administration. This fact in itself should not produce a concentration of the blood, although it might well lead to a reduction in the total volume of circulating blood. Almost invariably there was some hemolysis at the high temperatures. Bischoff and co-workers (1931) have reported considerable blood concentration in human subjects treated with "radio" frequency current.

The most striking and uniform result in the blood gas experiments (table 2) was the greatly diminished carbon dioxide content. Knudson and Schaible (1931) described this result in their dogs. Bischoff and co-workers (1930) (1931) published similar findings in their work on human subjects and with much lower body temperatures than the extremes which we report. In our experiments the carbon dioxide content of whole venous blood was reduced as much as 30 per cent (expt. 23). The smallest reduction (7.8 per cent) was noted in the case of the patient F.P. The percentage diminution in carbon dioxide content per degree C. rise in body temperature ranged from 2.2 (patient F.P) to 8.6 (expt. 23). Carbon dioxide capacity estimations show that in 5 of 6 experiments, the alkali reserve was diminished from 6.8 to 21.1 per cent. Only those samples taken after the administration of the anesthetic are considered, because it is likely that this procedure in itself affects both the carbon dioxide content and capacity. This influence, however, is probably in a direction opposite from what is ordinarily produced by high body temperatures. The intravenous injection of the sodium salt of a weak organic acid, such as sodium amytal, might conceivably result in a temporary alkalosis in which case the carbon dioxide content and capacity would be increased. We feel, however, that on this point our data are incomplete.

Not much regularity can be noted in the oxygen content figures as such, but if considered together with the oxygen capacity determinations, certain changes are apparent which are rather constant. For the purpose of comparison, the oxygen capacity, as determined after equilibration with alveolar air, is considered as representing complete hemoglobin saturation with oxygen. For all determinations made on blood drawn at a body tem-

perature greater than 41.0°C. the hemoglobin saturation was lowered 7 to 37 per cent. With smaller increments in the rise of body temperature, there were a few instances (expts. 25, 28, 31) in which the hemoglobin saturation was increased. Again we refer to the samples drawn after the injection of amytal.

In experiments 25 and in 27 to 31 inclusive, venous blood samples were obtained before the administration of amytal. These may be compared with the samples taken under complete anesthesia but just before the current was turned on. In the five of these for which the percentage hemoglobin saturation can be calculated, three show a decrease and two exhibit increases. Hence we cannot draw any conclusions regarding the effect of amytal in this respect. Control experiment 30 gives the following hemoglobin saturation figures over a period of $6\frac{3}{4}$ hours—83, 87, 79, 87 and 83 per cent.

The oxygen capacity determinations also show quite well the degree of concentration of the blood. A concentration of 58 per cent (expt. 23) is the maximum for the series, which is of the same order of magnitude as demonstrated by the hematocrit determinations.

DISCUSSION. The dog regulates his heat loss in a great measure by evaporation of water from the respiratory tract. Hence it is to be expected that, with such extremely high body temperatures as are reported here, the pulmonary ventilation should be greatly increased. It was shown in the second paper of this series (Nasset, 1932) that the ventilation may be increased fifteen fold. The two immediate results of this are 1, an accelerated loss of water by evaporation from the respiratory tract, and 2, a blowing off of carbon dioxide. Since the animals did not receive any fluids during the experiment, this loss of water must occur at the expense of the tissues. In several of the metabolism experiments it was noted that the temperature rose or remained constant for some time after the current was switched off. Balcar, Sansum and Woodyatt (1919) were able to produce very pronounced fevers in dogs by injecting large quantities of hypertonic sugar solutions intravenously. In one case the vaginal temperature was 52.5°C. at death. In a number of other animals the body temperatures were easily elevated to 44.0°C. They were able to show that the hypertonic solutions caused a dehydration of the tissues. They attribute the high body temperature to the lack of water and the subsequent inability of the tissues to lose the heat produced. Our results show a marked loss of water from the blood and this dehydration is probably a factor in maintaining the body temperature at a high level despite the fact that the current may be turned off. Flinn and Scott (1923) showed that normal dogs exposed to high environmental temperatures (45–50°C.) for one hour suffered a 6 per cent concentration of the blood. This, however, was not enough to maintain the body temperature at a high level once the animal was returned to his normal surroundings.

Judging from oxygen capacity determinations, the patient F.P., who took a large amount of fluid during the treatment, exhibited some concentration of the blood. It should be emphasized that the loss of water is one of the fundamental changes which occur in such treatments and in the therapeutic use of the high frequency current such losses should be compensated for by liberal administration of fluids.

Such gross changes in the water content of the blood make an appreciation of the total amount of the other constituents of the blood rather difficult. For instance, it has been shown (Nasset, Bishop and Warren, 1930) that the blood sugar in these extreme hyperthermias may fall to a level of 40 mgm. per cent. Now if this sample had suffered a 25 per cent concentration by evaporation of water, the sugar value in terms of milligrams per cent of the original volume would be 30. This illustration is not extreme, as is shown by the hematocrit and oxygen capacity values reported in this paper. By the same means of calculation the non-protein nitrogen of the blood, which in some experiments rose to 90 mgm. per cent, would be reduced to 77.5 mgm. per cent—a value still about double the average normal value.

The lowering of the carbon dioxide content of the blood has been repeatedly demonstrated in hyperthermias of various sorts. In their experiments with high frequency and "radio" frequency current, Bischoff *et al.*, found the total carbon dioxide content markedly reduced. They also found that simultaneously the pH was increased, and by using the constants for horse blood worked out by Van Slyke and co-workers (1924, 1928) they have calculated the increase in base bound by the blood proteins. From this computation they arrive at the conclusion that the alkali reserve of the blood either undergoes no change or a slight increase.

In dogs with temperatures up to 41.7°C. Knudson and Schaible found that the pH of the blood suffered no great change; above this temperature a marked acidosis ensued. Simultaneous chemical analyses of the blood revealed a very great increase in the concentration of lactic acid at the higher temperatures. Essentially similar changes were noted by Cajori, Crouter and Pemberton (1923) who studied the acid-base equilibrium in human subjects during heat treatment with the "electric bake." They determined the alkali reserve by direct absorption of carbon dioxide. From ammonia determinations on the urine and pH measurements on both urine and sweat, they concluded that a compensation for the alkalosis of the blood had occurred. Such responses to hyperthermia have also been noted by Adolph (1924), Koehler (1923) and others. Y. Henderson and Haggard (1918) produced excessive pulmonary ventilation in anesthetized dogs by artificial respiration and were able to show that both carbon dioxide content and capacity were very markedly reduced. In our experiments both the hyperthermia and the hyperventilation were carried to extremes

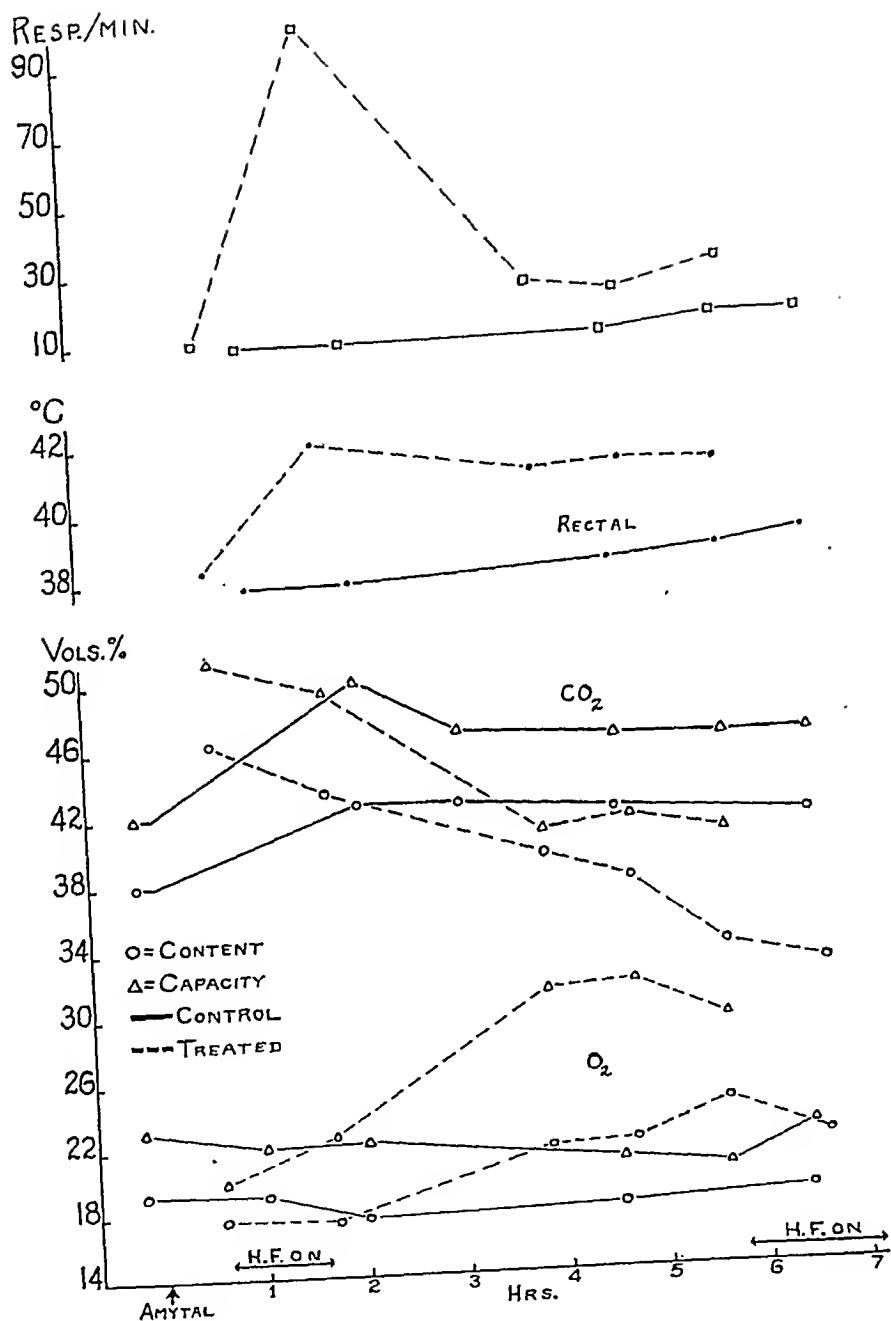


Fig. 1

with the result that both the carbon dioxide content and the alkali reserve, as measured by absorption of carbon dioxide, were decidedly diminished. The reduction in the ratio of carbonic acid to alkali bicarbonate at the

higher body temperatures (fig. 1) indicates indirectly an increase in the pH of the blood.

We are unable to say at present whether animals under our experimental conditions excrete an excess of fixed base in the urine. The possibility of a temporary combination of fixed base with lactic acid or the four-carbon chain fatty acids should not be overlooked in attempting to account for the reduction in alkali reserve. It has been shown in the earlier experiments of this investigation that the blood sugar may fall to rather low levels at the very high body temperatures. This may in a measure be the result of the inhibitory influence of amytal upon normal glycogenolysis (Olmsted and Giragossintz, 1931) but in any case the condition should lead to an increased combustion of fat. With the small amount of carbohydrate available for combustion and the possibility of a slight anoxemia, as indicated by the relative hemoglobin unsaturation at high temperatures, the condition would be favorable for the formation of beta-hydroxybutyric and aceto-acetic acids.

SUMMARY AND CONCLUSIONS

The experimental data show that in hyperthermia produced by high frequency electric current, the blood was concentrated 40 per cent or more. Below a body temperature of 41.0°C. the hemoglobin saturation of venous blood was increased; above this temperature it was diminished. The loss of water from the blood stream was accompanied, and probably partially compensated for, by a large storage of corpuscles in the spleen. The carbon dioxide content of venous blood was reduced as much as 30 per cent. The alkali reserve, as measured by carbon dioxide capacity, was decreased but to a lesser extent than the carbon dioxide content. It is suggested that this disturbance in the acid base relations of the blood is a consequence of three factors, namely, 1, hyperthermia; 2, hyperventilation, and 3, a greatly accelerated metabolic rate.

It is a pleasure to acknowledge the counsel of Prof. John R. Murlin and the assistance of Mr. J. W. Karr and Mr. H. Harrison.

BIBLIOGRAPHY

- ADOLPH, E. F. 1924. *This Journal*, lxvii, 573.
AUSTIN, J. H., G. E. CULLEN, A. B. HASTINGS, F. C. McCLEAN, J. P. PETERS AND D. D. VAN SLYKE. 1922. *Journ. Biol. Chem.*, liv, 121.
BALCAR, J. O., W. D. SANSUM AND R. T. WOODYATT. 1919. *Arch. Int. Med.*, xxiv, 116.
BISCHOFF, F., H. J. ULLMANN, E. HILL AND M. L. LONG. 1930. *Journ. Biol. Chem.*, lxxxv, 675.
BISCHOFF, F., M. L. LONG AND E. HILL. 1931a. *Journ. Biol. Chem.*, xc, 321.
BISCHOFF, F., L. C. MAXWELL AND E. HILL. 1931b. *Journ. Biol. Chem.*, xc, 331.

- BOURNE, W. 1926. *Brit. Journ. Anesthesia*, iv, 87.
- CAJORI, F. A., C. Y. CROUTER AND R. PEMBERTON. 1923. *Journ. Biol. Chem.*, lvii, 217.
- COOK, S. F. AND M. I. ROSE. 1930. *This Journal*, xcii, 240.
- FLINN, F. B. AND E. L. SCOTT. 1923. *This Journal*, lxvi, 191.
- HASTINGS, A. B., D. D. VAN SLYKE, J. M. NEILL, M. HEIDELBERGER AND C. R. HARINGTON. 1924. *Journ. Biol. Chem.*, lx, 89.
- HENDERSON, Y. AND W. H. HAGGARD. 1918. *Journ. Biol. Chem.*, xxxiii, 355.
- KNUDSON, A. AND P. J. SCHAIBLE. 1931. *Arch. Path.*, xi, 728.
- KOEHLER, A. E. 1923. *Arch. Int. Med.*, xxxi, 590.
- NASSET, E. S., F. W. BISHOP AND S. L. WARREN. 1931. *This Journal*, xcvi, 439.
- NASSET, E. S. 1932. *This Journal*, ci, 194
- OLMSTED, J. M. D. AND G. GIRAGOSSINTZ. 1931. *Journ. Lab. Clin. Med.*, xvi, 354.
- PERKINS, C. T. 1931. *New Eng. Journ. Med.*, ccv, 374.
- VAN SLYKE, D. D. AND J. M. NEILL. 1924. *Journ. Biol. Chem.*, lxi, 297.
- VAN SLYKE, D. D., A. B. HASTINGS, A. HILLER AND J. SENDROY, JR. *Journ. Biol. Chem.*, lxxix, 769.

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 101

JULY 1, 1932

No. 2

THE EFFECT OF LUMBAR SYMPATHECTOMY ON THE FLOW OF BLOOD IN THE FEMORAL ARTERY OF THE DOG

J. F. HERRICK, HIRAM E. ESSEX AND EDWARD J. BALDES

From the Division of Physics and Biophysical Research and the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota

Received for publication March 14, 1932

In recent years sympathectomy has been employed in an attempt to relieve certain pathologic conditions such as Raynaud's disease and other diseases in which the peripheral circulation of the extremities is inadequate. As observed clinically, definite improvement has followed such operations in some of these conditions. The improvement, in all probability, is due to an increased flow of blood to the extremities, owing to the release of the vessels from vasoconstrictor influence (1) (2). Indirect evidence of increased flow of blood to the extremities following lumbar sympathetic ganglionectomy in the dog has been reported by Sheard, Rynearson and Craig (5). A disease such as Raynaud's cannot be duplicated in animals, yet the effect of sympathectomy on the flow of blood to the extremities can be definitely determined. Since information of this nature might prove of value, and since quantitative measurements of flow of blood before and after sympathectomy on animals have not been reported, a series of experiments was performed in order to ascertain the quantitative alterations in flow in the femoral artery following lumbar sympathectomy in the dog. Herrick and Baldes have given a detailed description of the thermostromuhr method of Rein and its modification for measuring flow of blood.

In brief, the thermostromuhr method consists of heating the blood at a given place as it flows through the vessels, and of detecting the rise in temperature resulting from this heating. The blood is heated by applying electrodes which are connected to a high frequency circuit. The rise in temperature is measured indirectly by means of a differential thermocouple (the thermojunctions being placed equidistant above and below the place of heating) connected to a Zeiss loop galvanometer. This rise in temperature is a function of the flow of blood. Figure 1 illustrates the compendious unit called the diathermy thermo-element, containing heating

electrodes and thermojunctions, which is applied to the blood vessel in situ. The blood vessel is carefully isolated and freed from its connective tissues only so far as is necessary for insertion into the groove of the diathermy thermo-element. The wound is closed tightly and sufficient time is allowed for establishing heat equilibrium before any observations are made.

The initial experiments were acute and were performed under ether anesthesia. After the flow in each femoral artery was established, the entire chain of ganglia from the level of the second lumbar vertebra posteriorly to the end of the chain was removed (occasionally as was determined at necropsy the second lumbar ganglion was missed). Immediately following this operation the flow in each femoral artery was again measured. The results of these experiments were inconclusive since in some cases a

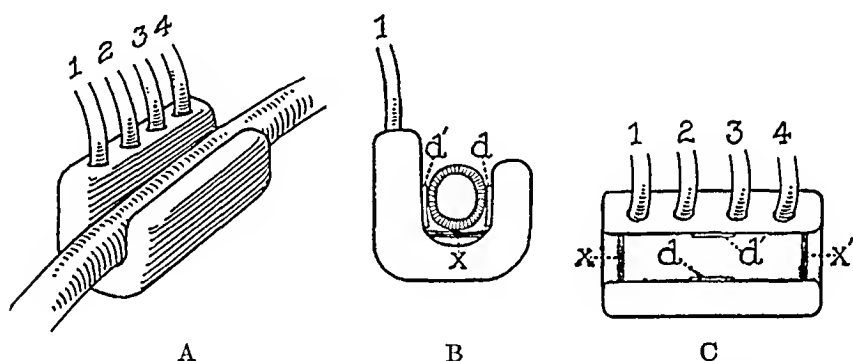


Fig. 1. Diathermy thermo-element. A shows its application on a blood vessel; B shows a cross section of the blood vessel as it fits against the electrodes (platinum plates) d and d' and on a thermojunction x . C shows the relative positions of plates and thermojunctions. x and x' are connected to the Zeiss loop galvanometer by leads 1 and 4. d and d' are connected to leads 2 and 3.

moderate increase in flow followed the sympathectomy, whereas in others significant changes were not observed. In certain experiments measurements were taken simultaneously with the removal of successive ganglia in the hope that this procedure would reveal the relative influence of each ganglion on the flow of blood to the limb. The results of these experiments were also inconclusive. It was suspected that ether anesthesia was responsible for the variable results. It was considered logical that the depth of anesthesia should materially influence the flow of blood to the limb. It is evident that in cases in which the anesthetic has produced complete relaxation of the vessels, sympathectomy should be incapable of causing much further dilatation. Support is given to these views by data presented later in this report.

Since a general anesthetic was considered inadvisable, subsequent ex-

periments were done under local anesthesia, the lumbar sympathectomy having been previously performed under ether anesthesia and sterile technic. The dogs were allowed to recover completely from the operation which usually required about two weeks. During this period the dogs were trained daily to lie quietly on the table and they thus became accustomed to the characteristic noise of the diathermy machine and also to the workers. At the end of the training period a comparative study of the flow in the femoral arteries was made, the artery on the intact side serving as the control. After the flow in one femoral artery was determined the same diathermy thermo-element was transferred to the other femoral artery and its flow established. In order to eliminate the personal equation involved in the observations, the sympathectomized side was unknown to the observer.

In a series of dogs of approximately the same weight the flow of blood was measured on the sympathectomized and intact sides under local anesthesia. The definite results of these experiments are in striking contrast to the inconclusive results obtained under general anesthesia. In this series the sympathectomized side showed a much greater flow of blood than the intact side in every instance; except in one case the flow in the former was twice as great (table 1).

As a result of the experiments described our interest was naturally aroused as to the difference in the flow in the right and left femoral arteries of normal dogs. For this study a series of apparently normal dogs was chosen and a comparison of the flow in the right and left femoral arteries was made first under local and then under ether anesthesia. The results of these experiments were surprisingly constant since the flow in the two arteries was found to be the same within the range of experimental error whether the experiment was done under local or general anesthesia. As was suspected after our early experiments, the flow under ether anesthesia showed a marked increase over that under local anesthesia in every instance (table 2).

The final experiments were devoted to a study of the relative influence of sympathectomy and general anesthesia on the flow in the femoral artery. As was indicated previously, changes in flow of blood were not detected after sympathectomy when a general anesthetic such as ether was used. It seemed probable that this might be due to the release of the vessels of the limb from vasoconstrictor influence. It was supposed that a general anesthetic brought about such complete relaxation of the vessels that any further dilatation which might be caused by sympathectomy could not be detected. In order to shed some light on this problem, the following experiments were done.

Two dogs were trained and a comparative study of the flow in the femoral arteries was made under local anesthesia. Immediately following

these observations, the dog was given a general anesthetic (ether) and the flow in each femoral artery again was determined. When the dog had recovered from the effects of this experiment a lumbar sympathectomy was performed as in previous instances. Two weeks after the operation, the flow in the femoral arteries was again studied under both local and general anesthesia. The results of these experiments demonstrate rather conclusively that the vasodilatation produced by deep general anesthesia is apparently as great as that caused by sympathectomy (table 3).

TABLE 1

Comparative blood flow in the femoral arteries of dogs following sympathectomy on the left side

DOG	WEIGHT	ARTERIAL FLOW PER MINUTE	
		Right femoral	Left femoral
	<i>kgm.</i>	<i>cc.</i>	<i>cc.</i>
1	13.6	215	377
14 days later	13.6	152	320
2	13.7	54	105
3	13.2	125	294
14 days later	13.2	131	293
4	11.2	165	383

TABLE 2

Comparative blood flow in the femoral arteries of normal dogs under local and general anesthesia

DOG	WEIGHT	ANESTHESIA	FLOW PER MINUTE	
			Right femoral	Left femoral
	<i>kgm.</i>		<i>cc.</i>	<i>cc.</i>
1	17.4	Local	89	82
		General	132	132
2	17.8	Local	80	77
		General	236	254
3	19.2	Local	64	63
		General	107	117

TABLE 3

Flow in femoral arteries in cubic centimeters per minute

	NORMAL		AFTER LEFT SYMPATHECTOMY	
	Local anesthesia	General anesthesia	Local anesthesia	General anesthesia
Right femoral.....	80	236	84	263
Left femoral.....	77	254	201	260

SUMMARY AND CONCLUSIONS

A series of studies has been made on the flow in the femoral artery of the dog. A comparison has been made of the minute volume flow in the right and left femoral arteries using both local and general anesthesia. After unilateral sympathectomy the flow was again measured. The results show that the flow is the same in both femoral arteries of the normal dog. Following unilateral sympathectomy the flow in the femoral artery on the sympathectomized side is about twice as great as that on the intact side. When

ether anesthesia is used in place of local infiltration there is not an outstanding difference between the intact and sympathectomized sides. It may be concluded that surgical ether anesthesia is almost as effective in producing vasodilatation as sympathectomy.

BIBLIOGRAPHY

- (1) BROWN, G. E. AND A. W. ADSON. *Amer. Journ. Med. Sci.*, 1925, clxx, 232.
- (2) BROWN, G. E. AND A. W. ADSON. *Arch. Neurol. and Psychiat.*, 1929, xxii, 322.
- (3) HERRICK, J. F. AND E. J. BALDES. *Physics*, 1931, i, 407.
- (4) REIN, H. *Zeitschr. f. Biol.*, 1928, lxxxvii, 394.
- (5) SHEARD, C., E. H. RYNEARSON AND W. MCK. CRAIG. *Journ. Clin. Invest.*, 1932, xi, 183.

THE MALE HORMONE

V. THE EFFECT OF THE MALE HORMONE AND THE ANTERIOR PITUITARY

CASIMIR FUNK AND BENJAMIN HARROW

*From the Casa Biochemica, Rueil-Malmaison, France, and the Department of Chemistry,
The College of the City of New York*

Received for publication March 24, 1932

Very soon after our initial experiments, in which we showed the presence of the male hormone in the male urine (1; see also 2-6), an elaborate series of tests on the effect of the hormone on old rats was begun. For this purpose, male rats from the Wistar Institute, as near the maximum age (plus or minus 3 years) were used. Each male rat was kept with two young female rats for a sufficiently long time to make certain that the male rat was sterile. The old rats were next divided into controls and experimental animals, and the latter were daily injected with the male hormone over a period of as many weeks as they continued to live. In no case could it be shown that the old, impotent rats could be made potent by male hormone injections.

In the meantime, the experiments of Smith and Engle, Ascheim and Zondek and others (7, 8) have made it evident that the anterior pituitary is responsible for stimulating the sex process in the female and in the male. As a preliminary to certain work we have in mind, the effect of the injection of the anterior pituitary hormone and the male hormone upon the seminal vesicles of the young rat has been studied.

EXPERIMENTAL. For purposes of comparison, we divided our rats, around a month old, into four groups: 1, controls; 2, injected with the male hormone; 3, injected with the anterior pituitary; 4, injected both with the male hormone and the anterior pituitary combined. In the first series of experiments, the rats were injected daily for six days, then killed and their seminal vesicles examined.¹ The very striking results obtained caused us to repeat these experiments, but this time *the animals were killed at the end of four days*. During the experimental period, each animal received a daily injection of 0.5 cc. of an extract of male hormone (the strength of which we shall presently discuss), and a twice daily injection of 0.3 cc. of the urine of pregnant women. The present uncertainty with regard to

¹ We are very much indebted to Dr. Alfred Plaut, of the Beth Israel Hospital, New York, for the histological examinations.

the potency of commercial extracts of anterior pituitary made us choose, for the time being, the urine of pregnant women itself.

We have described, in previous publications, our methods for preparing potent extracts of the male hormone. A brief description of the method employed in preparing the male hormone extract used in these experiments may here be given. The urine is strongly acidified and extracted with chloroform, under reflux, for eight hours. The chloroform portion is drawn off, the chloroform distilled, and the residue heated under reflux, for two hours, with 20 per cent sodium hydroxide. The product is repeatedly extracted with ether, the ether extract is evaporated, and the residue taken up in oil.² One cubic centimeter of the final product is equivalent to 10 cock units. The "cock unit" is defined as that amount of extract which will cause an increase in the size of comb and wattles of 20 per cent over the initial value in the course of 10 days. For each test five capons are employed. One "cock unit" represents the equivalent of about 125 cc. of urine.

From a number of experiments, all confirmative in character, we select the following as typical examples (compare with the photographs):

A. Control. Rat weighed 40 grams. At end of the experiment, the seminal vesicles were thin and glassy, 3.5 mm. long and 1.1 mm. wide. The ducts were thin.

A'. Control. Rat weighed 34 grams. The picture similar to A. The seminal vesicles were 4 mm. long and 1.5 mm. wide. (This one is not shown in the figure.)

B. Injected with male hormone. Rat weighed 35 grams. The seminal vesicles were not so thin and glassy as those of A and A'; they were 6 mm. long to 2 mm. wide. The ducts were thin and grayish.

C. Injected with male hormone. Rat weighed 30 grams. The vesicles were of the same appearance as B, and they were 5 mm. long and 1.5 mm. wide.

D. Injected with anterior pituitary. Rat weighed 40 grams. The seminal vesicles were grey and thin, 7.5 mm. long up to 3.5 mm. wide. They were conical in shape. The average width was less than 3.5 mm.

E. Injected with anterior pituitary. Rat weighed 33 grams. The seminal vesicles were 8 mm. long and 2.5 mm. wide.

F. Injected with the male hormone and the anterior pituitary. Rat weighed 41 grams. The seminal vesicles looked quite different; they were much thicker, and not glassy but opaque, having the ordinary yellowish pink color of organs. The seminal vesicles were 8 mm. long and 3 mm. wide, and very thick and folded. The ducts were considerably thicker than in the other groups (A-E).

² For technical assistance in these preparations we wish to thank Mr. Barnet Naiman, of the College of the City of New York.

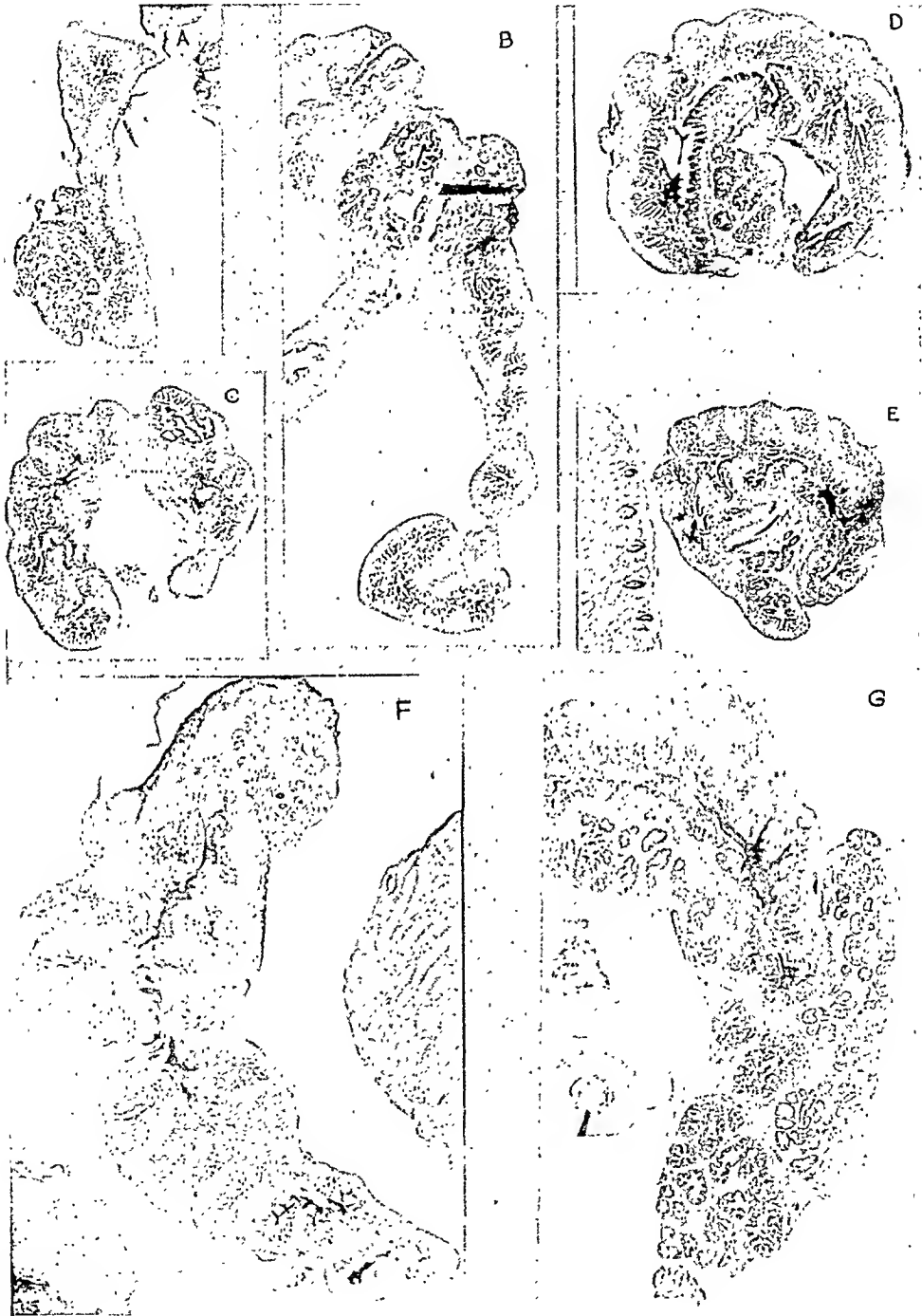


Fig. 1

G. Injected with the male hormone and the anterior pituitary. Rat weighed 35 grams. The seminal vesicles were 8.1 mm. long and 3.1 mm. wide, being very thick as in F.

DISCUSSION. The controls showed not only extremely small seminal vesicles (S. V.) but there was very little glandular development, and hardly any secretion. The S. V. of the animals injected with the male hormone were somewhat larger and they contained more secretion. As is well known, and as Moore and others (9, 10, 11) have shown, by injecting castrated rats with the male hormone, one can prevent the development of castration changes in the S. V. In fact, Moore and his co-workers have used this and a number of other methods for assaying the male hormone (12). Somewhat larger were the S.V. of the animals injected with the urine of pregnant women, and they showed a little more secretion. In this connection it may be of interest to note that Smith and Engel (7) found that the genital system of immature male rats is less responsive to anterior pituitary transplants than female rats, and that where mature male rats are used, no effect on the genital system is observed. However, Collip (13) found that by using an anterior-pituitary-like hormone obtained from human placenta, and injecting it into rats, 18 to 24 days old, for a period of 10 to 50 days, the S. V. showed appreciable increases in size. Somewhat similar results were obtained with adult male rats, $2\frac{1}{2}$ to 8 months old.

Where the rats received both the male hormone and the urine of pregnant women, the pictures of the seminal vesicles were outstanding: the organs were large, there were many ramifications of the glandular structure, and there was much secretion.

In comparison with the striking picture exhibited by the seminal vesicles, the other organs did not show much. There was no spermatogenesis in any of the animals. The ductus deferens did not show a characteristic reaction. The number of mitotic figures in the spermatogenes increased with the increase in size of the seminal vesicles.

BIBLIOGRAPHY

- (1) FUNK, C. AND B. HARROW. Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 325.
- (2) FUNK, C., B. HARROW AND A. LEJWA. Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 569.
- (3) FUNK, C., B. HARROW AND A. LEJWA. This Journal, 1930, xcii, 440.
- (4) FUNK, C. AND B. HARROW. Proc. Second International Sex Congress, 1930, p. 308.
- (5) FUNK, C. AND B. HARROW. Biochem. Journ., 1930, xxiv, 1678.
- (6) FUNK, C. AND B. HARROW. Proc. Amer. Soc. Biol. Chemists, 1931, vii, 70.
- (7) SMITH, P. E. AND E. T. ENGLE. Amer. Journ. Anat., 1927, xl, 159.
- (8) ZONDEK, B. AND S. ASCHHEIM. Klin. Wochenschr., 1927, vi, 248.
- (9) MOORE, C. R. AND T. F. GALLAGHER. This Journal, 1929, lxxxix, 388.
- (10) LOEWE, S. AND H. E. VOSS. Klin. Wochenschr., 1927, vi, 500.

- (11) DODDS, E. C., A. GREENWOOD, A. ALLAN AND E. J. GALLIMORE. *Biochem. Journ.* 1930, xxiv, 1031.
- (12) MOORE, C. R., T. F. GALLAGHER, D. PRICE AND W. HUGHES. *Amer. Journ. Anat.*, 1930, xlv, 39, 71, 109.
- (13) COLLIP, J. B., D. L. THOMPSON, M. K. MCPHAIL AND J. E. WILLIAMSON. *Canadian Med. Assoc. Journ.*, 1931, xxiv, 201.

FACTORS WHICH INFLUENCE THE FLOW AND PROTEIN CONTENT OF SUBCUTANEOUS LYMPH IN THE DOG

I. HEMORRHAGE AND HYPEREMIA*

FLORENCE W. HAYNES

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication March 26, 1932

Examination under different physiological conditions of the flow and protein content of lymph from the subcutaneous areas of the body is an important method of studying the nature of the tissue fluid and of measuring the interchange of fluid and protein between the capillaries and the lymph (Drinker and Field, 1931).

Although the literature contains a number of observations on the lymph flow from the thoracic duct, comparatively few (table 1) are to be found on lymph formed in the subcutaneous regions where the effects of digestion and respiration are relatively unimportant. While it is impossible to determine the total delivery of lymph in such areas, relative changes in lymph flow may be judged by cannulating a single large lymphatic vessel. Most previous observations of this kind include studies of the effect of active and passive hyperemia on the lymph flow (table 1). Paschutin (1872) and Emminghaus (1873) observed no increase in the lymph flow in the leg of the dog after active hyperemia due to nerve section, although the latter obtained a greater quantity of lymph after venous obstruction. Jankowski (1883) and Rogowicz (1885) later found an increase in the flow of lymph from the leg after cutting the sciatic nerve.

Unpublished observations by Field and Drinker have shown that in the development of a sterile inflammation due to heat the first phase of active hyperemia is accompanied by a slight increase in lymph flow and lymph protein. An extremely large increase in both of these is seen when the temperature of the water in which the foot is immersed reaches 60°C. At this point actual capillary injury apparently begins.

Because of lack of previous data a study has been made of the flow and protein content of lymph after hemorrhage. Observations have also been obtained after active hyperemia under conditions which allowed a definite and measurable increase of the arterial pressure and the blood flow to a part from which lymph was being collected. It was thought possible in this way to learn more about the passage of fluid and protein through the walls of the blood capillaries and lymphatics.

*Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Radcliffe College.

METHOD. Young dogs (15 to 27 kgm.), under Pentobarbital-Sodium, "Nembutal" (sodium-ethyl (1-methyl-butyl) barbiturate), anesthesia were used. The lymphatics accompanying the superficial veins at the ankle were cannulated. At the point chosen for cannulation two lymphatic trunks run beside the veins so that one may be tied and the other cannu-

TABLE 1
Conditions affecting the flow of subcutaneous lymph

ANIMAL	CONDITIONS OF EXPERIMENT	OBSERVER	YEAR	KIND OF LYMPH	CONDITIONS STUDIED	LYMPH FLOW		REMARKS
						Normal	After procedure	
						cc./10 min.	cc./10 min.	
Dog	Massage. Opium	Emminghaus	1873	Hind leg	Denervation (tibial and sciatic)	0.39	0.35	One experiment only. Study of cell counts
					Venous obstruction by a ligature around the leg	0.39	0.69	
					Venous obstruction directly on the vein	0.39	1.12	
Dog	Morphine. Passive motion and rest	Jankowski	1883	Hind leg	Denervation (sciatic)	3.73*	5.95	
Dog	Morphine. Curare	Rogowicz	1885	Hind leg	Turpentine inflammation			
					Denervation (sciatic)	0.15	0.40	
Dog	Passive motion	Winternitz	1895	Hind leg	Sciatic stimulation	19 mm.	14 mm.	
					Stimulation of vagi	46 mm.	75 mm.	
					Inflammation (turpentine)	0.97	0.96	
Dog	Curare and opium. Mechanical exercise	Paschutin	1872	Fore-leg	Denervation (medulla and cord)	1.08	0.80	
Dog	Nembutal or barbital. Massage	Field and Drinker	1931	Neck	Venous obstruction	0.72		
Horse	Permanent fistulas	Hamburger	1894	Neck	Plasmapheresis			
					Sterile inflammation			
					Pressure on jugular vein	2.88	7.00	
					Pressure on carotid artery	2.88	1.67	
					Feeding	2.88	8.79	
					Work	2.88	8.19	

* Irregular.

lated. In order to obtain a steady flow of lymph, the feet were kept in passive motion by loosely attaching the toes to a wheel which revolved approximately forty times per minute. In some experiments lymph was also obtained by massage from the large lymphatics draining the side of the head and neck. The lymph flowed into calibrated cannulas and was

removed with capillary pipettes at 10-minute intervals. The protein concentration was measured by a Zeiss refractometer, the same observer

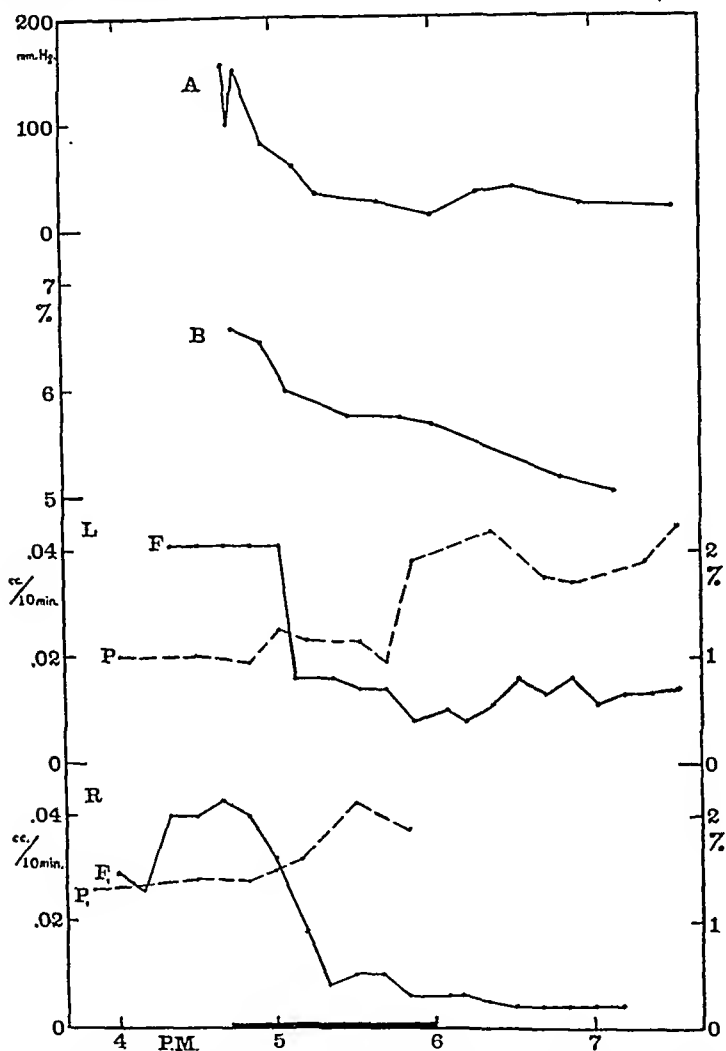


Fig. 1. The effect of hemorrhage on the arterial blood pressure, plasma protein, lymph flow and protein content of leg lymph. *A*, carotid blood pressure in millimeters of mercury; *B*, per cent protein in the blood plasma; *F* and *F*₁, lymph flow in cubic centimeters per 10 minutes in the left (*L*) and right (*R*) forelegs; *P* and *P*₁, per cent protein in lymph from the left (*L*) and right (*R*) forelegs. The solid line on the abscissa represents the time over which 1100 cc. of blood were removed.

making all the readings. In many experiments the blood pressure, protein content of the serum, and skin temperature of the foot were also followed.

Hemorrhage. In the first three experiments of this series, blood amounting to 1.7 to 2.7 per cent (380 to 625 cc.) of the body weight was removed

during a period of 5 to 10 minutes. The arterial pressure fell to about one-third of its normal value and recovered gradually during the next 2 to 3 hours. In the last three experiments, 100 to 200 cc. of blood were removed at intervals of from 10 to 30 minutes until 600 to 1100 cc. had been taken.

Figure 1 displays a typical experiment after severe hemorrhage. From a dog weighing 22 kgm., 1100 cc. of blood were removed in 200 cc. amounts over a period of one and one-quarter hours. Almost immediately after hemorrhage began the plasma protein, blood pressure and lymph flow fell markedly. The protein content of the lymph rose to a peak and then remained above the initial level. Similar changes were observed in all experiments of this series.

Active hyperemia. Since results in the literature on active hyperemia due to nerve section are inconsistent, a small group of experiments was performed in which the flow and protein content of leg lymph were determined before and after tying or cutting the saphenous and sciatic nerves. The skin temperature was measured by a thermocouple attached to the shaved surface of the foot.

Although a rise in skin temperature indicated a slight active hyperemia, no definite change in the flow of lymph nor its protein content was to be observed.

In order to obtain a definitely controlled increase in blood pressure and blood flow through the part from which lymph was collected, a perfusion pump of the type described by Richards and Drinker (1915) was used (fig. 2). The reservoir, 10, of the pump was filled continuously from the bottom with blood from the carotid artery. Details of the construction of the pump and valves are given in the paper of Richards and Drinker (1915). A membrane manometer was so placed that it would be connected to record either the normal blood pressure of the opposite leg or the pressure delivered by the pump to the perfused leg. Blood was pumped into the artery of the leg either at the ankle or the knee and returned through the animal to the venous reservoir. Aeration of the blood was thus obtained through the normal breathing of the dog. Before perfusion, heparin was injected through a cannula in the jugular vein. Heparin as received from the makers varies in its effects, some specimens lowering blood pressure seriously. The results reported are with non-toxic heparin which was shown during a control period to have no effect on the blood pressure and the flow and composition of lymph. Lymph was collected from the leg: 1, during a normal period; 2, after the injection of heparin; 3, during perfusion at a pressure equal to the normal pressure, and 4, during perfusion at an increased pressure. In some experiments the sciatic nerve was tied.

Figure 3 shows graphically the results of a typical experiment of this series. The arterial pressure was increased from 110 to 230 mm. of mercury with slight effect on the flow and protein content of the lymph.

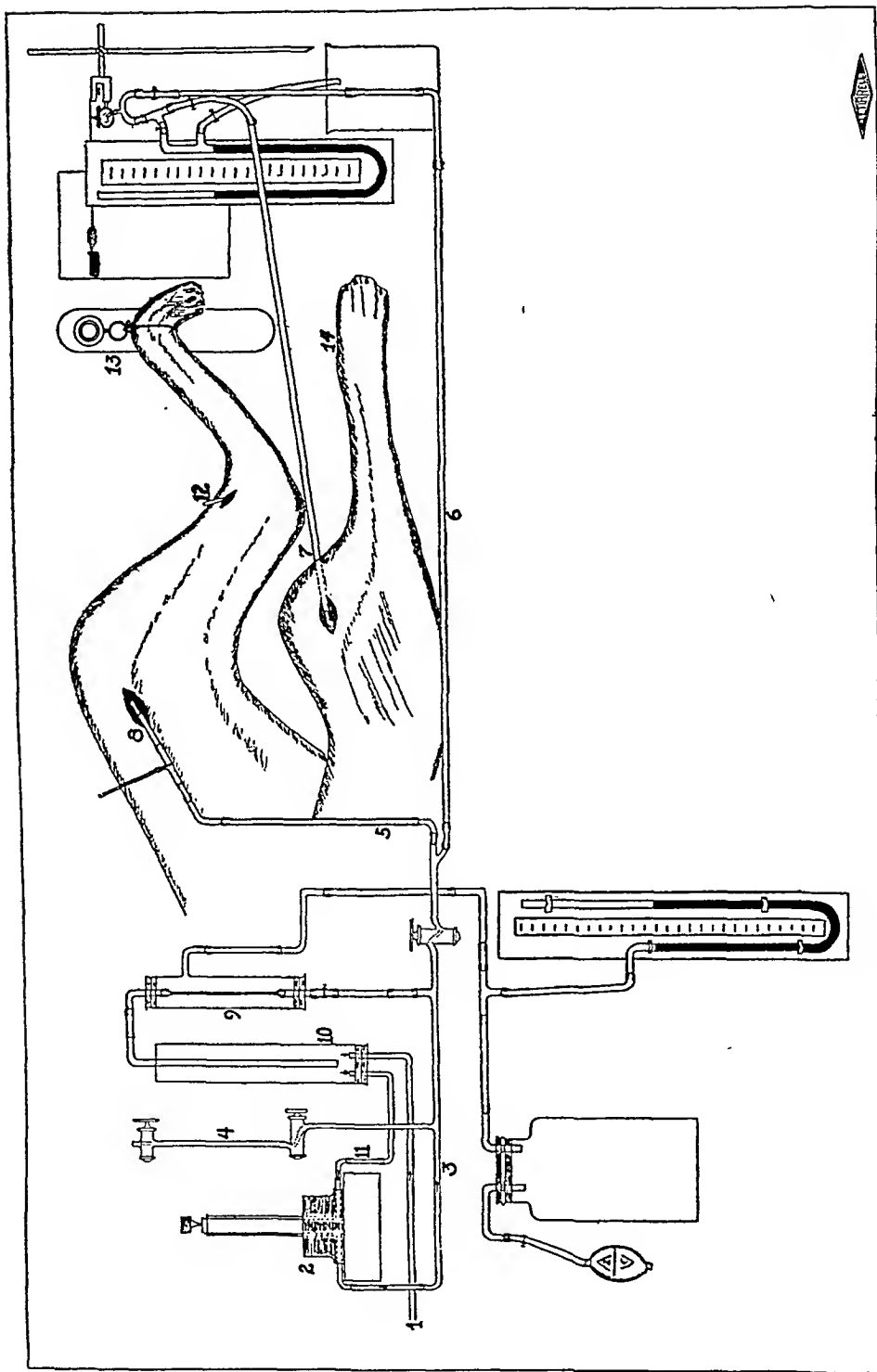


Fig. 2. Apparatus for perfusion and measurement of the arterial pressure in the legs of a dog. 1, inflow tube from carotid artery; 2, pump; 3, outflow from pump; 4, variable elasticity; 5, inflow to leg; 6, pump pressure connection; 7, arterial blood pressure connection; 8, arterial cannula; 9, capillary resistance, adjustment for excess pressure; 10, venous reservoir; 11, inflow to pump; 12, lymph cannula; 13, foot attached for passive motion; 14, opposite leg for measurement of normal blood pressure.

Tying the sciatic nerve at this increased pressure produced little change. A further increase of pressure to 290 mm. immediately caused an increased flow of lymph. In another experiment of this group, although the arterial pressure at the knee increased from 130 to over 300 mm. of mercury and the blood flow from 100 to 250 or 300 cc. per minute, the flow of lymph de-

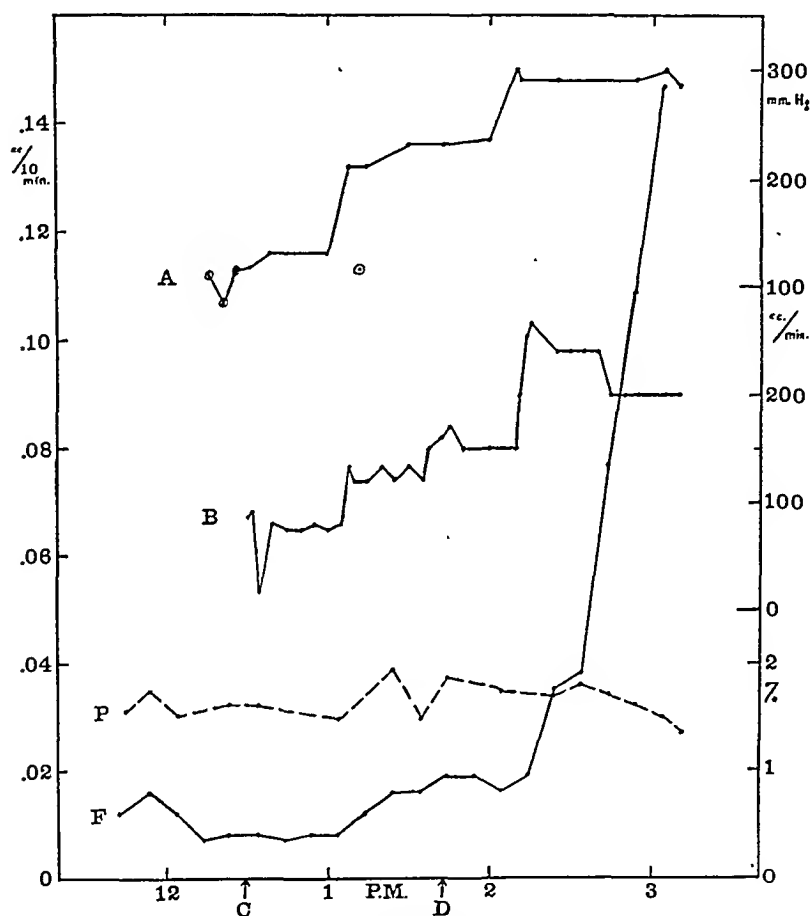


Fig. 3. The effect of increased arterial pressure and blood flow on the flow and protein content of leg lymph. A, arterial blood pressure of leg in millimeters of mercury (circles represent the arterial pressure without perfusion); B, blood flow through the leg in cubic centimeters per minute; P, per cent protein in lymph; F, flow of lymph in cubic centimeters per 10 minutes. At C perfusion of the leg was begun and at D the sciatic nerve was tied.

creased slightly and remained at a low level for over an hour. At this time partial venous obstruction was produced by tying a piece of rubber around the leg above the lymph cannula. Within ten minutes the flow had begun to increase and within a short time the protein content of the lymph decreased. This indicates, as have other experiments, that venous ob-

struction is more effective in causing an outpouring of fluid than increasing arterial pressure. In no case, whether the lymph flow increased or not,

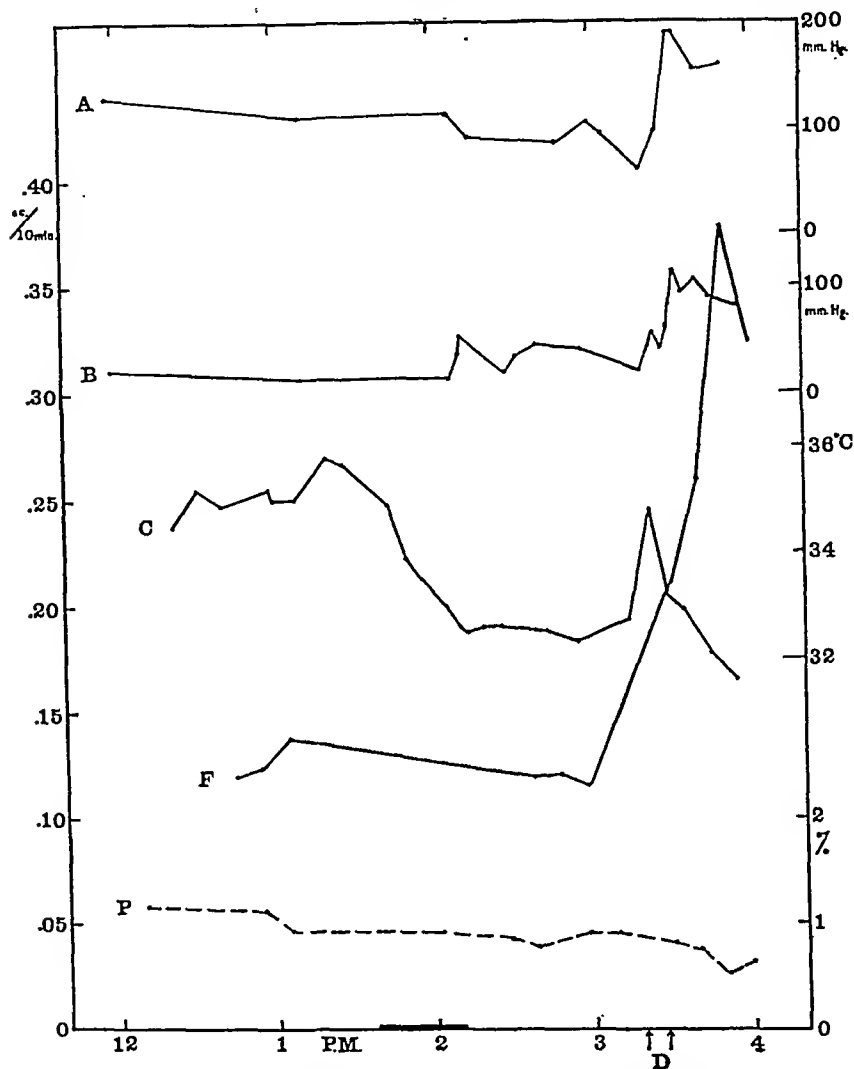


Fig. 4. The effect of arterio-venous anastomosis on the arterial and venous blood pressures, skin temperature and the flow and protein content of leg lymph. *A*, carotid blood pressure in millimeters of mercury; *B*, venous pressure (in millimeters of mercury) just above the lymph cannula; *C*, temperature of the skin of the foot; *F*, lymph flow in cubic centimeters per 10 minutes; *P*, per cent protein in lymph. The solid line on the abscissa represents the time taken to complete the anastomosis. At *D*, 90 mgm. of ephedrine were injected intravenously.

did the protein content of the lymph change significantly with an increase in arterial pressure.

In a later experiment (fig. 4), the venous pressure of the leg was increased

by an anastomosis between the femoral artery and vein. The venous pressure was measured at the ankle by a venous cannula which lead to a mercury manometer. The skin temperature was used as a criterion of the amount of blood going through the capillaries of the foot. After arterio-venous anastomosis the lymph flow and protein content were unchanged, but the arterial blood pressure and the skin temperature were low and the blood flow through the foot was probably slight. Pulsations could be felt in the part of the vein proximal to the anastomosis and a definite bruit could be heard over the veins at some distance below the union. When the arterial pressure was then increased by ephedrine the lymph poured out. At this time the protein fell slightly, showing that water was forced out faster than protein.

DISCUSSION. After hemorrhage the fluid and protein of the blood are quickly replaced. Hirota (1928) showed that if the hemorrhage is not too severe, more than 50 per cent of the amount of fluid lost is restored from the tissue fluid, usually in about half an hour. The protein of the blood has been shown by Morawitz (1906) to be made up to about half its normal value in the first three hours. As would be expected from such an inflow of fluid into the blood capillaries, the flow of lymph is decreased and the protein content increased. Thus, in hemorrhage as in venous obstruction and plasmapheresis, the lymph rapidly reflects changes in the tissue fluid. The amount of protein in the lymph does not appear large enough for any significant return of protein to the blood via the subcutaneous lymph. Calculations based on figure 1 show that the amount of protein coming out in the lymph per unit of time is about half its normal value after hemorrhage.

From a series of experiments on thoracic duct lymph, Starling (1894) concluded that intracapillary pressure is the chief factor in lymph production. In our experiments when the arterial pressure was altered through an isolated part from which lymph was collected, results indicate that arterial pressure and blood flow may be greatly increased without changing the flow and protein content of the lymph. Denervation has little effect on these results. This must be explained by the assumption that the resistance of the arterioles is sufficient to prevent undue augmentation of capillary pressure. It is only when the arteriolar resistance gives way under excessive pressure that the lymph flow suddenly increases. It may be assumed that venous obstruction was more effective in increasing capillary pressure and therefore lymph flow than arterial pressure. This observation agrees with Rous' idea of a gradient of permeability toward the venous end of the capillary. McMaster and Hudack (1932) have recently shown that this gradient is accentuated and broadened in scope by moderately increasing the venous pressure, so that transudation takes place abundantly from the venules. The permeability of the portion of the capil-

lary web near the arterioles is increased only when venous pressure approximates that in the arteries.

The fact that arterio-venous anastomosis did not increase the lymph flow may probably be accounted for by the fact that blood was shunted back by the vein and the foot was poorly supplied with blood. This is indicated by the low level of the skin temperature. When the arterial pressure was sufficiently increased by ephedrine so that the blood flow was resumed and the effect of increased venous pressure alone was obtained, the lymph flow increased.

SUMMARY

1. Hemorrhage decreases the flow of subcutaneous lymph and increases its protein content.

2. Active hyperemia due to arterial perfusion of the dog's leg under increased pressure has little effect on the lymph from the part until the arterial pressure has become nearly three times its normal value.

The writer wishes to express her gratitude to Dr. Cecil K. Drinker, who suggested the problem on which this work is based, for advice and help, as well as to Dr. Madeleine E. Field for assistance in performing the experiments.

BIBLIOGRAPHY

- DRINKER, C. K. AND M. E. FIELD. 1931. *This Journal*, xcvi, 32.
EMMINGHAUS, H. 1873. *Ludwig's Arb. a. d. physiol. Anstalt zu Leipsig*, viii, 51.
FIELD, M. E. AND C. K. DRINKER. 1931. *This Journal*, xcvi, 378.
HAMBURGER, H. J. 1894. *Zeitschr. f. Biol.*, xxx, 143.
HIROTA, K. 1928. *Journ. Biochem.*, ix, 87.
JANKOWSKI, K. W. 1883. *Virchow's Arch. f. path. Anat.*, xciii, 259.
MCMASTER, P. D. AND S. HUDACK. 1932. *Journ. Exper. Med.*, lv, 417.
MORAWITZ, P. 1906. *Beitr. z. chem. Physiol. u. Path.*, vii, 153.
PASCHUTIN. 1872. *Ludwig's Arb. a. d. physiol. Anstalt zu Leipsig*, vii, 197.
RICHARDS, A. N. AND C. K. DRINKER. 1915. *Journ. Pharm. Exper. Therap.*, vii, 467.
ROGOWICZ, N. 1885. *Pflüger's Arch.*, xxxvi, 252.
STARLING, E. H. 1894. *Journ. Physiol.*, xvi, 224.
WINTERITZ, R. 1895. *Arch. f. exper. Path. u. Pharm.*, xxxvi, 212.

FURTHER OBSERVATIONS ON THE RAPIDITY OF PASSAGE OF SUBSTANCES FROM BLOOD TO LYMPH IN THE DOG*

FLORENCE W. HAYNES

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication March 26, 1932

Observations on subcutaneous as well as thoracic duct lymph show that many substances pass rapidly from the blood to the lymph. Rous and Gilding (1929) injected a very diffusible dye, brom phenol blue, intravenously in rabbits and cats over a period of one minute and found the mesenteric lymphatics distended with blue fluid within 15 seconds after injection, a vivid expression of the great permeability of the capillaries in this region and of the ease with which material in the tissue spaces reaches lymphatics. Less diffusible dyes, as trypan blue and vital red, injected intravenously in dogs, have been observed in the thoracic duct lymph after 8 to 12 minutes (Meyer-Bisch and Lampe, 1924; Keith, Rowntree and Geraghty, 1915; and Harris, 1920). Smith (1925) was able to detect vital red in the thoracic duct lymph within 2 to 3 minutes after intravenous injection.

Substances of larger molecular weight have also been found to pass rapidly from the blood into this lymph. Petersen, Levinson and Hughes (1923) observed that injected hemoglobin went from the blood to the thoracic duct lymph in 12 to 15 minutes. Osato (1921) states that true solutions as well as colloids (indigo carmine, phenolsulphonaphthalein, foreign protein and antibodies) introduced into the blood regularly appear in the thoracic duct lymph in 4 to 5 minutes after injection. Field and Drinker (1931a, b) working on lymph from subcutaneous lymphatics of dogs showed by intravenous protein injections as well as by experiments on sterile inflammation, venous obstruction and plasmapheresis, that subcutaneous lymph promptly reflects alterations in the constituents of the serum. In none of these experiments, however, have accurate measurements been made of the time taken for substances to pass from the blood stream into the subcutaneous lymph. Such measurements are significant in comparison with determinations on the thoracic duct lymph since the latter comes largely from the liver and intestines, the capillaries of which are known to be exceptionally permeable to protein.

*Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Radcliffe College.

Many of the experiments on lymph formation now in progress in this laboratory have shown a very rapid equalization of concentration of substances between blood and lymph. The work of Rous and his associates has provided data on the rate at which dyes of different degrees of diffusibility leave the capillaries. The time elapsing between their appearance in the capillaries and in the subcutaneous lymph has been considered of importance in indicating the degree of extravascular circulation which is constantly in progress. In order to measure this time a very diffusible dye, brom phenol blue, has been employed, and the interval between intravenous injection, the first coloration of the skin indicating capillary

TABLE 1
Passage of substances from blood to subcutaneous lymph

SUBSTANCE	DOSE	MOLECULAR WEIGHT	KIND OF LYMPH	NUMBER OF DETERMINATIONS	TIME OF APPEARANCE IN LYMPH	AVERAGE TIME	REMARKS
					minutes	minutes	
Brom phenol blue	45 to 55 cc. of 3 to 4 per cent solution	670	Cervical	3	2.1 to 3.0	2.7	Skin and tissue appeared blue in one-half minute
			Foreleg	3	3.5 to 9.0	6.3	
			Hind leg	3	4.0 to 11.0	6.5	
			Mesenteric	1		2.5	
Vital red	50 cc. of 2 per cent solution	1,096	Cervical	3	1.5 to 3	2.2	One experiment after pitressin and one after ephedrine
			Hind leg	2	4.5 to 11	7.8	
Egg albumen	100 cc. of 5 \pm per cent solution	34,500 (Svedberg and Nichols, 1926)	Cervical	4	9 to 14	12	
			Hind leg	3	11 to 13	13	
Hemoglobin (dog)	100 cc. of 6.5 \pm per cent solution	66,800 (Svedberg and Fåhræus, 1926, horse Hb)	Cervical	4	37 to 48	44	
			Foreleg	1	32	32	

escape, and the appearance in cannulated lymphatic trunks has been recorded. To obtain an idea of the speed with which substances of different molecular weights pass into the lymph, similar experiments have been performed with a slowly diffusible dye and with two proteins of widely different molecular size.

METHODS AND RESULTS. The dogs used were anesthetized with Pentobarbital-Sodium, "Nembutal" (sodium-ethyl (1-methyl-butyl) barbituate), intraperitoneally. The lymphatics of the lower leg and of the cervical region were cannulated as previously described (Haynes, 1932). Substances such as brom phenol blue,¹ vital red and hemoglobin were injected

¹ A specially purified product obtained from Hynson, Westcott and Dunning.

into the jugular or femoral vein, and the lymph collected from the cannulas in capillary tubes every one-half or one minute thereafter. By comparing such tubes with tubes of normal lymph the first trace of color could be observed. Egg albumen could be accurately detected in lymph by a micro precipitation test.² Usually slight pressure below the tip of the cannula was necessary to force lymph in the lymph vessel into the cannula. The arterial blood pressure and the protein content of the lymph were measured before and after injection.

Brom phenol blue was found to be non-toxic when the hydrogen ion concentration was adjusted to that of the blood. Vital red, hemoglobin and egg albumen injections were also innocuous as judged by the constancy of the arterial blood pressure and of the protein content of the lymph. Lymph flow did not change significantly.

The results, summarized in table 1, show that dyes such as brom phenol blue and vital red appeared in lymph from the neck in approximately $2\frac{1}{2}$ minutes and in the lymph from the legs in 6 to 8 minutes. The skin and mucous membranes appeared blue in less than 30 seconds.

Egg albumen could be detected in the cervical lymph in 9 to 14 minutes after the beginning of injection and in the lymph of the hind leg in 13 minutes. Hemoglobin, although much more difficult of detection, appeared in the subcutaneous lymph from 30 to 45 minutes after injection.

DISCUSSION. The data in table 1 indicate that the time required for the passage of substances into the lymph is increased with an increase in the molecular weight of the substance. The great difficulty of detecting the color of hemoglobin in the lymph probably accounts in part for the relatively late appearance of this material.

The use of vital red is significant since Harrop and Waterfield (1930) have claimed that in the dye method of measuring the blood volume part of the vital red diffuses into the lymph spaces, thus making the method less accurate than the other methods. The present experiments confirm the observations of Smith (1925) on thoracic duct lymph and make it obvious that the dye must begin to pass out of the blood capillaries almost immediately.

Of the total times determined in these experiments, the time for the injected substance to be carried by the blood to different parts of the body may be considered roughly as of the order of 10 to 20 seconds. We observed that the skin and mucous membranes were diffusely stained 30 seconds after the beginning of an injection of brom phenol blue. It may thus be considered that dissolved substances in the tissue spaces may get into small lymphatics and be carried to large collecting trunks in the

² Anti-egg white rabbit serum of a titre of 1-100,000 was supplied by Dr. Walter Bauer.

legs in less than 6 minutes. In the neck region the time is only about 2 minutes.

SUMMARY

NON-TOXIC SUBSTANCE INTRAVENOUSLY INJECTED	NATURE OF SUBSTANCE	AVERAGE TIME OF APPEARANCE IN	
		Cervical lymph	Leg lymph
		minutes	minutes
Brom phenol blue.....	Very diffusible dye	2.7	6.4
Vital red.....	Slowly diffusible dye	2.2	7.8
Egg albumen.....	Protein of relatively small molecular weight	12	13
Hemoglobin.....	Substance of large molecular weight	44	32

These experiments as well as experiments on substances such as histamine, intravenously injected, also suggest a rapid interchange of material between blood and subcutaneous lymph.

The writer wishes to express her gratitude to Dr. Cecil K. Drinker and to Dr. Madeleine E. Field whose advice and assistance were invaluable during the course of these experiments.

BIBLIOGRAPHY

- FIELD, M. E. AND C. K. DRINKER. 1931a. This Journal, xcvi, 40.
 1931b. This Journal, xcvi, 378.
 HARRIS, D. T. 1920. Brit. Journ. Exper. Pathol., i, 142.
 HARROP, G. A. AND R. L. WATERFIELD. 1930. Journ. Physiol., lxx, p. xxxii.
 HAYNES, F. W. 1932. This Journal, ci, 223.
 KEITH, N. M., L. G. ROWNTREE AND J. T. GERAGHTY. 1915. Arch. Int. Med., xvi, 547.
 MEYER-BISCH, R. AND W. LAMPE. 1924. Zeitschr. f. d. gesamt. exper. Med., xliii, 761.
 OSATO, S. 1921. Tohoku Journ. Exper. Med., ii, 325, 465.
 PETERSEN, W. F., S. A. LEVINSON AND T. P. HUGHES. 1923. Journ. Immunol., viii, 323.
 ROUS, P. AND H. P. GILDING. 1929. Journ. Exper. Med., l, 189.
 SMITH, H. P. 1925. Bull. Johns Hopkins Hosp., xxxvi, 325.
 SVEDBERG, T. AND R. FÄHREUS. 1926. Journ. Amer. Chem. Soc., xlviii, 430.
 SVEDBERG, T. AND J. B. NICHOLS. 1926. Journ. Amer. Chem. Soc., xlviii, 3081.

THE ACCUMULATION OF LACTIC ACID IN EXCISED LIVER TISSUE

JOHN HALDI

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan

Received for publication March 28, 1932

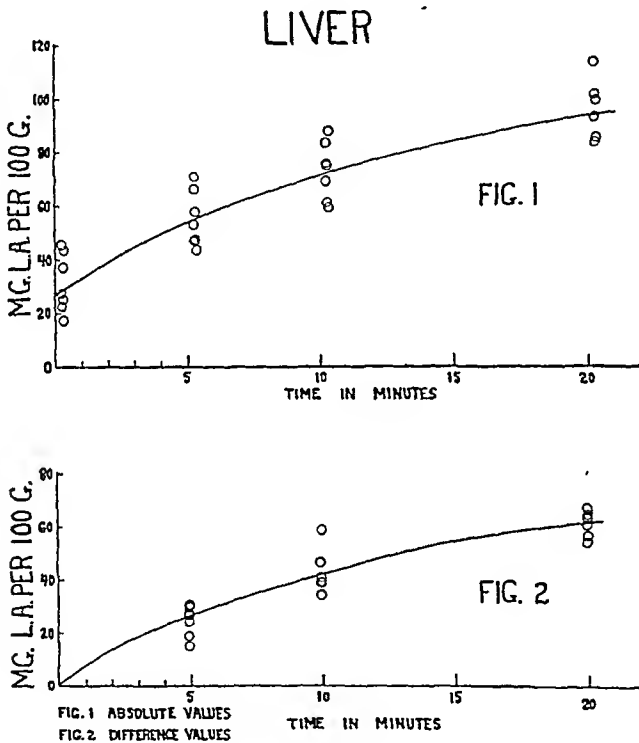
The initial lactic acid content and the rate of lactic acid accumulation in excised dogs' liver during a twenty minute period have been determined as supplementary data to a previous report (Haldi, 1932) on the accumulation of lactic acid in excised brain, kidney, muscle and testicle. The procedure was essentially the same as in the former experiments. Immediately after decapitation of the animal one lobe of the liver was quickly removed and a portion of the tissue, approximately 3 to 6 grams, sliced off and plunged into liquid air. Fifteen to eighteen seconds elapsed from the moment the blade of the guillotine struck the animal's head until the tissue was immersed in liquid air. Three other pieces of tissue of approximately equal weight were taken from the same lobe, incubated 5, 10 and 20 minutes respectively at 38°C. and then frozen in liquid air.

The results of the experiments are represented in figures 1 and 2. The initial lactic acid content in 7 experiments varied between 17.1 and 45.7 mgm. per cent with an average of 31.3 mgm. per cent. After 5, 10 and 20 minutes' incubation the liver contained an average of 54.7, 73.0 and 95.6 mgm. per cent respectively.

The increase in lactic acid was determined by subtracting the initial content in milligrams per cent from the amount present after incubation. The average increase in lactic acid content after 5, 10 and 20 minutes' incubation of the tissue was 23.4, 41.7 and 64.3 mgm. per cent.

In one experiment unusually high values were obtained which are not represented in the figures. The initial lactic acid content was 63.2 mgm. per cent and at the end of 5, 10 and 20 minutes' incubation the tissue contained 96.9, 129.6 and 154.0 mgm. per cent or an increase of 33.7, 66.4, and 90.8 mgm. per cent respectively. These values were omitted in constructing the curves because of the conditions of the experiment. The animal was extremely vivacious and when brought to the laboratory continuously ran around the room in a lively manner for several minutes before it was decapitated. When brought up to the guillotine it had to be forcefully restrained because of its efforts to break loose from the attendant. It was therefore thought that the high lactic acid values might not represent normal variations but might have been due to the activity of the animal.

Comparison of the curves in figures 1 and 2 reveals a wider variation in the absolute values at various intervals than in the difference values. This might possibly be explained on the basis of the observations by Himwich, Koskoff and Nahum (1928, 1929) that the liver removes lactic acid from the blood. The lactic acid concentration of the blood, and, in all probability, the functional activity of the liver varies with different animals. On the plausible assumption that various livers contain a different amount of lactic acid absorbed from the blood, we might expect to find wide variations in the initial lactic acid content of the liver which would be maintained



in the absolute values throughout the experiment. These variations however would not show up in the curve of difference values which represents only the rate of lactic acid formation in liver tissue.

SUMMARY

The initial lactic acid content and the rate of lactic acid accumulation in excised dog's liver has been determined.

The initial content (15 to 18 seconds after decapitation) varied between 17.1 and 45.7 mgm. per cent with an average of 31.3 mgm. per cent.

The average increase was 23.4, 41.7 and 61.3 mgm. per cent after 5, 10 and 20 minutes' incubation.

Unusually high values were obtained in one experiment. Owing to the conditions of the experiment it is impossible to decide whether these values represented a normal variation or should be attributed to the activity of the animal previous to decapitation.

The absolute values showed wider variations than difference values. An explanation is suggested on the basis of the observations that liver tissue absorbs lactic acid from the blood.

BIBLIOGRAPHY

- HALDI, J. 1932. This Journal, xcix, 702.
HIMWICH, H. E., Y. D. KOSKOFF AND L. H. NAHUM. 1928. Proc. Soc. Exper. Biol. and Med., xxv, 347.
1929. Journ. Biol. Chem., lxxxv, 571.

PREVENTION OF "CASTRATION CELLS" IN THE ANTERIOR PITUITARY OF THE MALE RAT BY ADMINISTRATION OF THE MALE SEX HORMONE

JOHN D. REESE AND MORVYTH McQUEEN-WILLIAMS

From the Department of Anatomy, and the Institute of Experimental Biology, University of California

Received for publication March 28, 1932

Since Biedl (1912) reported the discovery by Zacherl of the castration cell in the anterior hypophysis of the rat, numerous articles of a corroboratory nature have appeared until now the phenomenon is a matter of common knowledge. That the "castration cell" was not an entirely new cell type, however, was later established by Addison (1917) who showed that it is really the basophile normal to the anterior lobe which has undergone a characteristic modification after gonadectomy. He states that the castration reaction is discernible at the end of the first week.

That castration changes in the anterior hypophysis might be inhibited by substitution of the endocrine principles from the gonads has occurred to several workers. Fichera (1905) thought that the hypophyses of three capons that he had treated with saline extracts of cock testes approached the normal. Schleidt (1914), upon investigation of the hypophyses of Steinach's feminized male and masculinized female rats, reported that heterologous implants prevented castration changes in that form. Nukariya (1926) stated that in castrated rats treated with saline extracts of the rat epididymis and testis the basophiles typical of castration were somewhat less numerous than in the untreated animals castrated for a like period. Lehmann (1927) describes inhibition of the castration picture in castrated rats implanted with rat and human gonads and also those treated with extracts of human gonads. Fluhmann and Kulchar (1931) were unable to prevent the development of castration cells in the female rat by the use of "amniotin (Squibb)."

MATERIAL AND METHOD. The work of Moore, Gallagher, Koch, and others has yielded the male sex hormone in a rather highly purified state. The fact that the preparations of these workers have undergone far more than a cursory study and that they may be standardized by four methods (Moore, Hughes and Gallagher, 1930) suggested to us that a study of the effect of these extracts on the rat pituitary in castration might prove of much interest. Through the courtesy of Doctor Gallagher a small amount of extract derived from male urine (method as yet unpublished) and of

proven potency, was made available for this study. This material containing $17\frac{1}{2}$ bird units per cubic centimeter was injected subcutaneously in doses of 0.25 cc. twice daily into two adult castrated male rats. Injection started immediately after castration and was continued for twenty days.

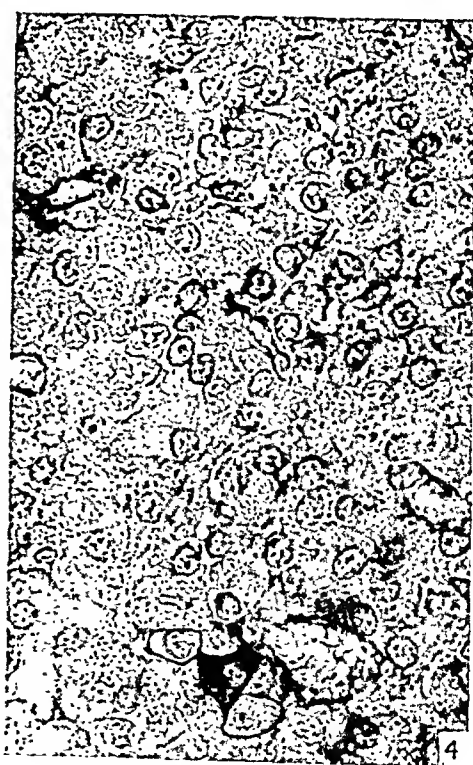
Following essentially the method of Gallagher and Koch (1929), we undertook to prepare an extract of bull testes. The method used may be briefly outlined as follows: The ground testicular tissue¹ was extracted with four volumes of 95 per cent ethyl alcohol, the ground tissue filtered off, and the filtrate evaporated in vacuo. The residue was extracted with benzene, the precipitate discarded, and the benzene evaporated in vacuo. The residue from the benzene was extracted with acetone, the acetone evaporated, and the acetone-soluble fraction dissolved in petroleum ether. The petroleum ether preparation was then shaken in a separatory funnel with a 70 per cent alcohol solution. The 70 per cent alcohol fraction was concentrated and taken up in ether; the ether was then evaporated and the ether-soluble material taken up in olive oil. Injections of this preparation in 0.25 cc. doses twice daily for twenty days were given to five adult castrated male rats. Six other castrated male animals of the same age were used as controls. Two of these were injected with corresponding doses of the aqueous fraction which had been separated from the ether-soluble material in the last step. Another rat was injected with equal doses of the same olive oil as had been used for dissolving the ether-soluble material. Four rats of the same age were kept as normal controls.

In the series of rats given bull testes extract, treated castrates, castrate controls, and normals were fixed in the same container, carried through all procedures together, and finally mounted on the same slides. The histological methods employed for all hypophyses may be described briefly: After fixation in Helly's fluid for one hour at a temperature of 35° to 37°C., the gland is washed in distilled water, dehydrated as far as 70 per cent alcohol and left overnight. Dehydration must then be completed, after which the tissue is cleared in oil of bergamot and imbedded in paraffin.

Plate I. Explanation of figures. Photomicrographs of anterior hypophyses of adult male rats. Preparations were fixed in Helly's fluid and stained with orange G and aniline blue ($\times 600$).

1. Anterior hypophysis of rat castrated for twenty days.
2. Anterior hypophysis of normal untreated rat. Ink outlines denote cells shown in figures 5 and 9.
3. Anterior hypophysis of twenty-day castrated rat receiving injections of human male urine extract during the twenty days after castration.
4. Anterior hypophysis of twenty-day castrated rat receiving injections of bull testes extract during the twenty days after castration. Ink outlines denote cells shown in figure 6.

¹ Approximately 15 kilograms of fresh bull testes were used.



Serial sections are cut at 7.5 micra. As it has been the experience in this laboratory that hematoxylin-cosin preparations are entirely unsatisfactory from the standpoint of cell differentiation in the rat hypophysis, we have resorted to a modification of the Mallory orange G-aniline blue technique which has proved satisfactory. Details of the technique are as follows: After going through xylol and the higher concentrations of alcohol, the slide is left 90 minutes in iodized 70 per cent alcohol to remove mercuric chloride crystals. It is then passed through alcohols of decreasing concentration to water and thence to a slightly acidified solution of two per cent aqueous orange G where it remains for 20 minutes. The slide is drained of excess dye and placed in one per cent phosphomolybdic acid for four minutes when it is again drained. Then for a period of 12 seconds the slide is moved back and forth in a five-tenths per cent aqueous solution of aniline blue. Next it is rinsed in 95 per cent alcohol, dehydration completed in 100 per cent alcohol and after xylol clearing, the tissue is mounted in xylol balsam.

RESULTS. In order that the following experimental results may be entirely comprehensible we may here briefly summarize the changes in the rat hypophysis during the first three weeks after castration. At the end of the stated period one finds in the castrate pituitary a marked increase in the number of basophiles. In addition to basophilic cells not exceeding the normal in size and depth of staining, one sees cells which exceed normal diameters. In general, these large cells fall into two classes. The very largest cells stain lightly with aniline blue. The smaller ones take a deeper stain and possess an exceedingly prominent macula which is usually central (figs. 1 and 8). The prominence of the macula, *m*, is characteristic of the basophile after castration. In the hypophysis of the normal rat the macula is not as often seen in the basophile and its prominence in the cytoplasm is by no means as well marked as after gonadectomy.

Previous to this experiment Reese studied the cell types in the anterior

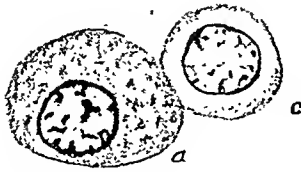
Plate II. Explanation of figures. Camera-lucida drawings of cells of anterior hypophyses of adult male rats. Preparations were fixed in Helly's fluid and stained with orange G and aniline blue. Abbreviations: *a*, acidophile; *b*, basophile; *b'*, large deeply staining basophile with prominent macula, *m*, typical of twenty-day castrated rats; *b''*, very large lightly staining basophile typical of twenty-day castrated rats; *m*, macula; *c*, chromophobe. ($\times 1600$)

5. Anterior hypophysis of normal untreated rat. Group of cells marked on right upper corner of figure 2, plate I.

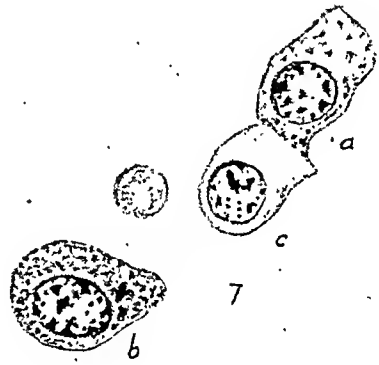
6, 7. Anterior hypophyses of two twenty-day castrated rats receiving injections of bull testes extract during the twenty days after castration. Figure 6 shows the group of cells marked on figure 4, plate I.

8. Anterior hypophysis of rat castrated for twenty days.

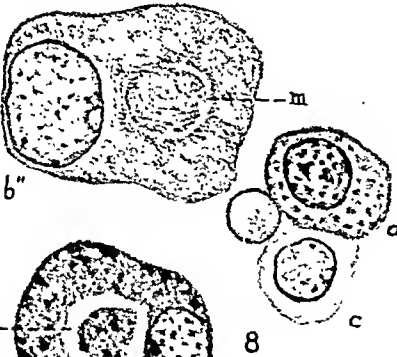
9. Anterior hypophysis of normal untreated rat. Group of cells outlined in center of figure 2, plate I.



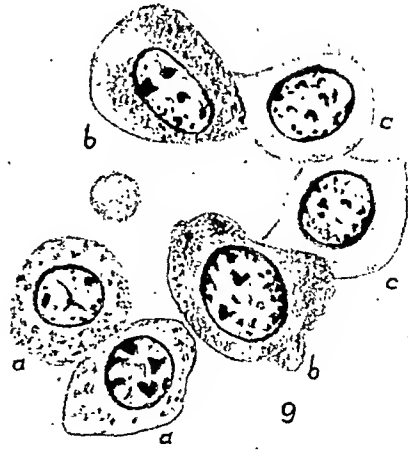
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pituitary of the female rat after castration, and reports having found that in the female there is approximately a threefold increase in the number of basophiles twenty days after castration. His work was based on differential counts of more than 4000 cells made on representative fields in the pituitaries of both normal and castrate types.

In the present experiment where only male rats were under consideration, cell counting was undertaken by McQueen-Williams for the purpose of determining the relative number of the three cell types prevailing in typical fields in the hypophyses of normal and castrate animals. These fields were not picked at random but were those that seemed to express the predominant characteristic of the sections studied. The total number of cells counted for each group of animals amounted to over 10,000. The results would seem to indicate that in the case of the adult male rat there is almost a twofold increase in the number of basophiles in the hypophysis twenty days after castration.

The two animals which were injected with the urine material from Doctor Gallagher's laboratory showed in the seminal vesicles the picture given by Moore, Hughes and Gallagher (1930). In these treated castrates the involution of the secretory epithelium of the seminal vesicle and anterior lobe of the prostate incident to castration was minimal and this epithelium was in no way comparable to that of the twenty day castrate. Similar results were obtained in the case of the rats treated with bull testes extract. An interesting fact pertains to the relative weights in this last series: The average weight of the seminal vesicle in the castrates was 140 mgm. as compared with 636 mgm. for the treated castrates and 710 mgm. for the normal rats.

A study of the anterior lobe of the pituitary in the two rats treated with the urine derivative showed in this gland a marked absence of the basophile so typical of the castrate animal. For comparison of the two conditions see figures 1 and 3. The basophiles are smaller and far less numerous in the injected animals than in the controls castrated for the same period, and the prominence of the macula and its position in the central portion of the cytoplasm which is so characteristic of the castrate is seldom observed in the treated castrate and is likewise rare in the normal.

The five castrates treated with the ether-soluble fraction from the bull testes exhibited a picture in the hypophysis the principal aspects of which were identical with those treated with the urine material. Here again the basophile changes typical of castration were not to be found (figs. 4, 6 and 7). On the other hand, controls injected with the aqueous residue after ether extraction of the alcohol-soluble fraction in amounts volumetrically equivalent to the lipid-soluble bull testes extract showed marked castration changes. This was also true of the animal injected with olive oil alone.

CONCLUSION

The morphological changes in the anterior lobe of the male rat hypophysis incident to castration may be prevented by the administration of extracts containing the active principle of the male gonads. The pituitary gland of the castrate male rat may be similarly affected by treatment with a derivative of human male urine.

Acknowledgments. We wish to express our gratitude to Dr. Samuel Lepkovsky for his expert aid in the preparation of the testes extract, to Dr. T. F. Gallagher for so kindly placing his preparation at our disposal, to Dr. Olive Swezy for suggestions regarding the appended illustrations, and to Dr. M. E. Simpson and Dr. H. M. Evans for advice, encouragement, and material assistance in the undertaking of this problem and the carrying of it to fulfillment.

BIBLIOGRAPHY

- ADDISON, W. F. 1917. Journ. Comp. Neurol., xxviii, 1.
BIEDL, A. 1912. Innere Sekretion, Berlin, ii, 108.
FICHERA, G. 1905. Arch. Ital. d. Biol., xliii, 405.
FLUHMAN, C. F. AND G. V. KULCHAR. 1931. Proc Soc. Exp. Biol. and Med., xxviii, 417.
GALLAGHER, T. F. AND F. C. KOCH. 1929. Journ. Biol. Chem., lxxxiv, 495.
LEHMANN, J. 1927. Pflüger's Arch., ccxvi, 729.
MOORE, C. R., W. HUGHES AND T. F. GALLAGHER. 1930. Amer. Journ. Anat., xlv, 109.
NUKARIYA, S. 1926. Pflüger's Arch., ccxiv, 697.
SCHLEIDT, J. 1914. Zentralbl. f. Physiol., xxvii, 1170.

THE ANTERIOR PITUITARY SEX HORMONE IN THE BLOOD AND URINE OF RATS

FREDERICK E. EMERY

From the Physiology Department, University of Buffalo

Received for publication March 30, 1932

It is now well established in a few species of animals that during pregnancy the anterior pituitary sex hormones, or hormones with similar action, are secreted in much larger amounts than during the non-pregnant state. This increase in the amount of the hormones in the blood and urine of pregnant animals has been studied especially in women and mares and to some extent in other mammals. It seems that in rats the test is negative during pregnancy (Snyder and Wislocki, 1931). However, in castrated rats the results may be positive according to Zondek (1930) who, in a footnote, calls attention to a positive test he obtained by injecting urine. The present article deals with the anterior pituitary sex hormones in the blood and urine of albino rats in respect to sex and castration.

The test used was similar to those in general use and depends upon an increase in weight of the ovaries and uterus and the appearance of cornified cells in the vagina of immature rats. Young females about 25 days old and weighing 36 to 40 grams were taken from the mother, marked and started on the test. Subcutaneous injections were made twice daily when urine was used, while with blood the morning injection was subcutaneous and the evening injection intraperitoneal. The injections continued for a period of 4 to 15 days. Three or four days after the last injection the animals were killed, the ovaries and uterus, cut from the vagina just anterior to the cervix and with the Fallopian tubes intact, were dissected free from connective tissue and weighed in a closed container. This method of weighing has been used in the laboratory for some time (Emery, Bash and Lewis, 1931) and, therefore, the error in weight due to dissecting out these organs was small.

Blood. During the past two years a large number of rats have been killed for various purposes. These animals with a known history have afforded an excellent opportunity to obtain blood in sufficient amounts to carry out the tests here described. Table 1 shows the effects of serum from three types of donors on the weights of the ovaries and uteri and changes in the vagina of the recipients. It is interesting that the anterior pituitary sex hormones were not found in pregnant rat serum even though as much as

25 cc. was given to an immature rat whereas 14 to 16 cc. of castrated male serum caused the ovaries and uterus to enlarge and the vagina to open in full oestrus. In this respect pregnant rat serum differs from that of pregnant women and mares. The negative results from serum of normal male and female rats previously reported (Emery, Bash and Lewis, 1931) were again confirmed.

Although not shown in table 1, the blood serum of castrated multiparous female rats has been found to be potent. The amount of blood available in this group has been small and, therefore, only a few tests have been made. These tests show that the potency of castrated female serum is similar to that of castrated male serum as shown in table 1. These data would lend support to the view that castration in rats results in a hypersecretion of the anterior pituitary sex hormones and not simply a storage of the hormones in the gland as Evans and Simpson (1929) described. In humans where the ovaries were removed or in certain cases with various menstrual

TABLE 1

Rat serum from three kinds of donors was injected into immature female rats (recipients). Body weight in grams, ovaries and uteri in milligrams.

RECIPIENTS						DONORS
Number of rats	Body weight	Ovaries weight	Uteri weight	Number of vaginas open	Number in oestrus	Average amount and kind of serum injected
20	45	20	60	18	15	19 cc. castrated male
9	50	15	40	None	None	26 cc. normal male
5	48	12	34	None	None	17 cc. pregnant
15	57	15	40	None	None	None (controls)

disturbances, positive results, similar to those here described for rats, have been reported by Fluhmann (1929) and Mazer and Hoffman (1931). In fact in cases of hydatidiform mole and malignant chorionepithelioma the amount of the hormones excreted is greater than that excreted during normal pregnancy (Mack and Catherwood, 1930).

Urine. The rats were placed in a wire cage with a large glass funnel beneath, which drained the urine into a test-tube containing ether. Screen wire in the funnel kept the feces and hair from falling into the test-tube. Toxic substances were further reduced by shaking with ether as described by Böhne (1931). In these experiments one volume of urine was shaken with two volumes of ether, then filtered. The ether in some cases was removed by bubbling air through it at room temperature; in other cases heating in a water bath at 70°C. removed all traces of the ether. This simple method although almost entirely preventing fecal contamination did not remove all the toxic substances from the urine. It was, therefore, necessary to limit the daily dose to 2 or 3 cc. and continue the injections over a

10 to 15 day-period in order to give a total dose of 30 to 40 cc. of urine to each immature rat. Even these large doses gave negative results. Since this is about 10 times the threshold dose for pregnant human urine, it would seem unlikely that larger doses of rat urine given in this way would show positive results. An attempt was, therefore, made to make an extract of the rat urine using the alcohol precipitation method of Frank (1931). The results so far have all been negative even when the extract from 100 cc. or more of urine was given to each immature rat. Extracts made from the urine of pregnant, castrated female and castrated male rats were tried but no evidence of the hormones was found. These urine extracts are often toxic as others also have reported (Hill and Parkes, 1930). These toxic substances and the small amount of urine available are factors which hinder the preparation of the hormone in concentrated form from rat urine.

TABLE 2

Urine from three types of rats (donors) was injected into immature female rats (recipients). Body weight in grams, ovaries and uteri in milligrams. Compare these data to controls, table 1.

RECIPIENTS						DONORS
Number of rats	Body weight	Ovaries weight	Uteri weight	Number of vaginas open	Number in oestrus	Average amount and kind of urine injected
10	48	10	27	None	None	24 cc. castrated male
10	64	14	41	None	None	27 cc. castrated female
8	64	12	44	None	None	28 cc. pregnant

The abundance of the pituitary sex hormones in pregnant human urine (using this rat test, we have obtained positive results with less than 5 cc. of pregnant human urine) is quite in contrast to the findings with rat urine. In table 2 tests from urine of pregnant, castrated female and castrated male rats are given. It is clearly seen that the ovaries and uterus were not enlarged; in fact the weights are slightly below the normals shown in table 1. It was thought probable that toxic substances in the urine may have retarded the growth of the ovaries and uterus as well as body weight. On the other hand, rats while being injected daily with urine readily respond to pituitary grafts, thus showing the urine injections did not retard or noticeably inhibit the action of the anterior pituitary sex hormones when present in amounts large enough to stimulate growth and luteinization of the follicles.

DISCUSSION. The amount of anterior pituitary sex hormones found in the blood serum of castrated male and castrated female rats is considerably less than the amount present in pregnant human serum. Trivino (1926), Siddall (1928), and Fluhmann (1929 and 1930) have reported

positive results in immature and adult mice with 3-5 cc. of pregnant human serum. This compares with 14 cc. of castrated rat serum which is the minimal amount that will produce enlargement of the uterus and the appearance of cornified cells in the vagina of immature rats (table 1). It is interesting that with threshold doses of this hormone, either given as pituitary grafts (Emery, Bash and Lewis, 1931) or injected as blood serum (table 1), the ovaries may increase only a few milligrams in weight whereas the percentage increases in weight of the uterus is considerably more. This seems to show that a small enlargement of the follicles as represented by the increased weight of the ovaries can stimulate a large growth of the uterus. Although the ovaries may be decreased in size accompanied by a positive vaginal reaction (Cole and Hart, 1930), there is no definite proof that the anterior pituitary sex hormones can produce growth of the rat's uterus independent of the ovaries.

Larger doses, 25 to 30 cc., of castrated male serum caused the ovaries of immature rats to enlarge to 100 milligrams or more. These ovaries were full of large corpora lutea and resembled those produced by pituitary grafts. This is further evidence that the ovarian stimulating hormones in the blood of castrated rats are similar if not identical to those of the pituitary gland.

It was disappointing to find that the pregnant serum of rats did not contain the pituitary sex hormones characteristic of some of the other mammals. In addition to the positive results with pregnant human serum cited above Cole and Hart (1930) have discovered in the serum of pregnant mares a most remarkably high concentration of this hormone. They have obtained an increase in the weight of rat's ovaries with as little as $\frac{1}{16}$ cc. of serum; this is about three hundred times the concentration found in castrated rat serum.

A fair amount of the hormone in the blood and lack of it in the urine of castrated rats is interesting because pregnant human urine is loaded with the hormone while pregnant human serum does not seem to be so potent. It was expected that the urine of rats would be found positive for the pituitary sex hormone and that a comparison could be made with the amount in the serum. Such a study would throw light on the threshold of the kidney for this hormone in the several types of rats studied. In some animals the kidney threshold probably does determine to some extent the output of the hormone in the urine (Frank, 1931). Aside from pregnant human urine positive results have been reported from the urine of monkeys by Aschheim (1930). In contrast to this, negative results have been reported in several animals, as cat, dog, rabbit, rat, sow, mouse, cow and elephant (Aschheim, 1930; Leonard, 1931; Snyder and Wislocki, 1931). These variations of the hormone occur not only in the urine of different species of animals but even pituitary grafts from pregnant cows (Bacon, 1930) and pregnant sows

(Wolfe, 1931) have failed to show an increase in potency. It is likewise peculiar that pituitary glands from dogs had little or no action when grafted into monkeys (Allen, 1928). These variations although difficult to understand serve as a stimulus to further research.

SUMMARY

1. The amount of anterior pituitary sex hormones in the blood and urine of several types of rat donors was tested by injecting immature female rats.
2. Positive results, as represented by enlargement of the ovaries and uterus and signs of oestrus, were obtained with castrated male and castrated female sera.
3. The hormone was not found in serum of normal males, normal females, multiparous or pregnant females.
4. Tests with urine collected from normal males, castrated males and females and pregnant rats were all negative.
5. Extracts made from urine were likewise negative.

BIBLIOGRAPHY

- ALLEN, E. 1928. *Anat. Rec.*, xxxix, 315.
- ASCHHEIM, S. 1930. *Amer. Journ. Obst. and Gynecol.*, xix, 335.
- BACON, A. R. 1930. *Amer. Journ., Obst. and Gynecol.*, xix, 352.
- BÖHNE, C. 1931. *Klin. Wochenschr.*, x, 210.
- COLE, H. H. AND G. H. HART. 1930. *This Journal*, xciii, 57; xciv, 597.
- FLUHMAN, C. F. 1929. *Journ. Amer. Med. Assoc.*, xciii, 672, 1930. *Amer. Journ. Obst. and Gynecol.*, xx, 1.
- FRANK, R. T. 1931. *Journ. Amer. Med. Assoc.*, xcvi, 1852.
- EMERY, F. E., P. W. BASH AND W. R. LEWIS. 1931. *Proc. Soc. Exp. Biol. and Med.*, xxix, 42.
- EVANS, H. M. AND M. E. SIMPSON. 1929. *This Journal*, lxxxix, 371.
- HILL, M. AND A. S. PARKES. 1930. *Proc. Roy. Soc. London B*, cvii, 30.
- LEONARD, S. 1931. *This Journal*, xcvi, 412.
- MACK, H. C. AND A. E. CATHERWOOD. 1930. *Amer. Journ. Obst. and Gynecol.*, xx, 670.
- MAZER, C. AND J. HOFFMAN. 1931. *Journ. Amer. Med. Assoc.*, xcvi, 19.
- SIDDALL, A. S. 1928. *Journ. Amer. Med. Assoc.*, xc, 380; xci, 779.
- SNYDER, F. F. AND G. B. WISLOCKI. 1931. *Anat. Rec.*, xlviii, 34.
- TRIVINO, F. G. 1926. *Klin. Wochenschr.*, v, 2022.
- WOLFE, J. M. 1931. *Amer. Journ. Anat.*, xlviii, 391.
- ZONDEK, B. 1930. *Arch. f. Gynäkol.*, cxliv, 156.

METABOLISM DURING GROWTH IN A COMMON PIGEON

OSCAR RIDDLE, THEODORA C. NUSSMANN AND FRANCIS G. BENEDICT

From the Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and the Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.

Received for publication March 31, 1932

The effects of age upon the basal metabolism, particularly in the period from birth or hatching to maturity, are known in very few animals. Fairly adequate data for the human were obtained by DuBois (1916), Benedict (1919) and Benedict and Talbot (1921). Deighton (1924) and Wood (1926) obtained the essential facts for the pig; Mitchell, Card and Haines (1927) supplied important data for the fowl; and Brody and Ragsdale (1930) provided significant details for the calf. It is remarkable that the newborn of all these four species have an initial low metabolism which rather quickly rises to a maximum and then declines during maturity and adult life. It is found that this same situation reappears in the results of the present study. A few measurements have been made on the metabolism of nestling birds, including pigeons. Pembrey (1897) found that, though the chick is homothermic at hatching, the young pigeon begins to give evidence of this property only at 7 or 8 days after hatching, and only at 15 days was its heat regulation as well developed as that of the newly hatched chick. Leichtentritt (1919) and Plaut (1921) found a relatively low metabolism in young nestlings which Groebbels (1927) failed to confirm in pigeons, probably because of inadequate data and of failure to recognize the very short duration of this low metabolism in this species.

In the present study the metabolism during six early stages of growth, besides an early (107 days) and late (164 days) phase of "adolescence" and a still later stage representing adult life, was measured in a small and well-marked race of common pigeons known as tipplers. On these nine age-groups 130 measurements were made. The earliest stage used was 3 days after hatching, which represents 21 days from the beginning of embryonic development. Because of other and current studies on these birds it is desirable here to calculate age from the beginning of development, not from hatching. Those who wish to think of age as dating from hatching may do so by subtracting 18 days from the values stated in this paper. At 21 days, and for more than 10 days thereafter, the rate of growth in the pigeon is extraordinarily rapid (see table 1), and the metabolism measurements made at these two earliest stages are therefore of exceptional interest. Other

measurements were made at 41-day and 43½-day stages when growth is still rapid but distinctly less so than in the preceding periods.

All these very early growth stages (to 50 days) were measured on non-fasting birds (excepting 3 birds aged 43 days, as noted later), because it was further sought (successfully on 10 birds) to measure the same individuals at several successive growth stages. No special preliminary preparation was used in the case of these youngest birds; they were taken directly from their nests to the metabolism chamber; they remained in the metabolism chamber at 30° for approximately 6 hours; they had been gorged with food shortly before beginning the test, and at its end they usually had much food in their crops; the weight of the crop contents was accurately estimated, and this quantity subtracted from the total weight of the bird. All birds more than 50 days old, however, were fasted for 24 hours and subjected to those several conditions of preparation and measurement which Benedict and Riddle (1929) have earlier described as necessary to basal measurements in the pigeon. These conditions, we would like to emphasize, include a 24-hour fast (begun, in birds weighing 200 to 260 grams, with 8 grams' grain and 8 grams' water in the bird's crop) spent in a large glass cage kept at 30°C.; with measurements made at 30°C., at night, in completely darkened chambers; and with activity recorded by kymograph. Since the sex of young pigeons can not be recognized no attempt has been made to analyze our data on the basis of sex. We have, however, assured ourselves that the two sexes are fairly distributed in all age-groups.

Somewhat arbitrarily, yet almost necessarily, we selected 30°C. as the environmental temperature at which to make all measurements. This temperature seemed the lowest at which we could keep the youngest birds comfortable in the metabolism chamber—even with a protecting bed and covering of absorbent cotton. Birds of the 29-day stage were provided with this bed, but with less covering. Most, though not all, of these very young birds seemed comfortable when removed from the metabolism chamber; the rectal temperatures then obtained, and the relative quiet during measurement, also indicated that the covering supplied to these birds enabled them to remain essentially normal and comfortable in the 30° environment. We do not suppose, however, that the temperature used was equally agreeable and suitable to these many stages of a rapidly changing organism. Indeed, we know that the birds of the 21-day stage, while normally covered in their nests by their parents, are usually given an environmental temperature in excess of 35°C. (their covering there being the feathers and skin of the parent); also that the "critical" temperature of the adult pigeon is approximately 30°C. and measurements made on them at 33° or 35° give higher metabolism values than are obtained at 30°C. (unpublished data). Surface was calculated from the formula: $S = 10 \times W^{\frac{2}{3}}$. Here S = square centimeter, and W = body weight in grams.

EXPERIMENTAL RESULTS. A summary of results is given in table 1. The very youngest stage (21 days, 3 days after hatching), though not fasted and in extremely rapid growth, has a metabolism not much higher than that of the fasting adolescent bird (107 to 164 days). Though crammed with food this youngest stage gives a respiratory quotient which indicates that little or no carbohydrate is being burned; and this corresponds well with the observation that at this stage these young are fed almost exclusively on "crop-milk"—a secretion containing much fat, some protein and no carbohydrate. On the other hand, the other and next stage of extremely rapid growth (29 days) is indicated as having the highest metabolism (1284

TABLE 1

Summary of data from measurements of the respiratory metabolism during growth in a common pigeon (tuppler)

All measurements made during March-June, at 30°C.

AGE (FROM BEGINNING OF DEVELOPMENT)	BODY WEIGHT (END OF TEST)	CONDITION	R.Q. (AVERAGE)	CALORIES PER SQUARE METER PER 24 HOURS	NUMBER OF TESTS
<i>days</i>	<i>grams</i>				
21	29.6*	Not fasting	0.768	773	13*
29	127.3	Not fasting	0.991	1,284	24
41	231.1	Not fasting	0.987	1,125	12
43.5	247.6	Not fasting	0.980	1,050	12
43.3	221.8	Fasting 24 hours	0.745	922	3
56	211.8	Fasting 24 hours	0.726	727	16
80	237.0	Fasting 24 hours	0.731	719	14
107	236.8	Fasting 24 hours	0.707	737	8
164	247.7	Fasting 24 hours	0.703	632	9
439	254.3	Fasting 24 hours	0.760	638	15

* In this group 13 pairs, or 26 birds, were used since a single bird was too small for satisfactory measurement.

calories per square meter per 24 hours) attained during the entire life cycle, and at this and the two next following stages chiefly carbohydrate is burned—a fact shown by respiratory quotients of about 0.99. At the age of 41 and 43½ days the total metabolism is definitely reduced below the 29-day value. It is at 30 to 45 days that the plumage becomes a very effective agent for the conservation of heat, and this may be concerned in the marked and progressive decrease in heat production observed at this period. When 30 days old the young are not continuously covered by their parents, except in cold weather; at 40 days (22 after hatching) the young have already deserted the nest and, though they seek and require other means of shelter, they are no longer brooded by the parents.

The values found for age-groups which were fasted before measurement indicate, in general, a small progressive decline in the metabolism from

the 56-day stage to the 435-day stage; between 56 and 107 days, however, such a decline is not indicated by our data. We have attempted a measure of the extent to which the very high metabolism at the 43-day stage is influenced by the non-fasting condition of this group. Three such birds were measured first in the non-fasting condition and again 24 hours later after fasting under the precise conditions used with the older groups of birds. The results of this special test must be described although they are partly

METABOLISM DURING GROWTH IN THE PIGEON (TIPLERS, AT 30° C)

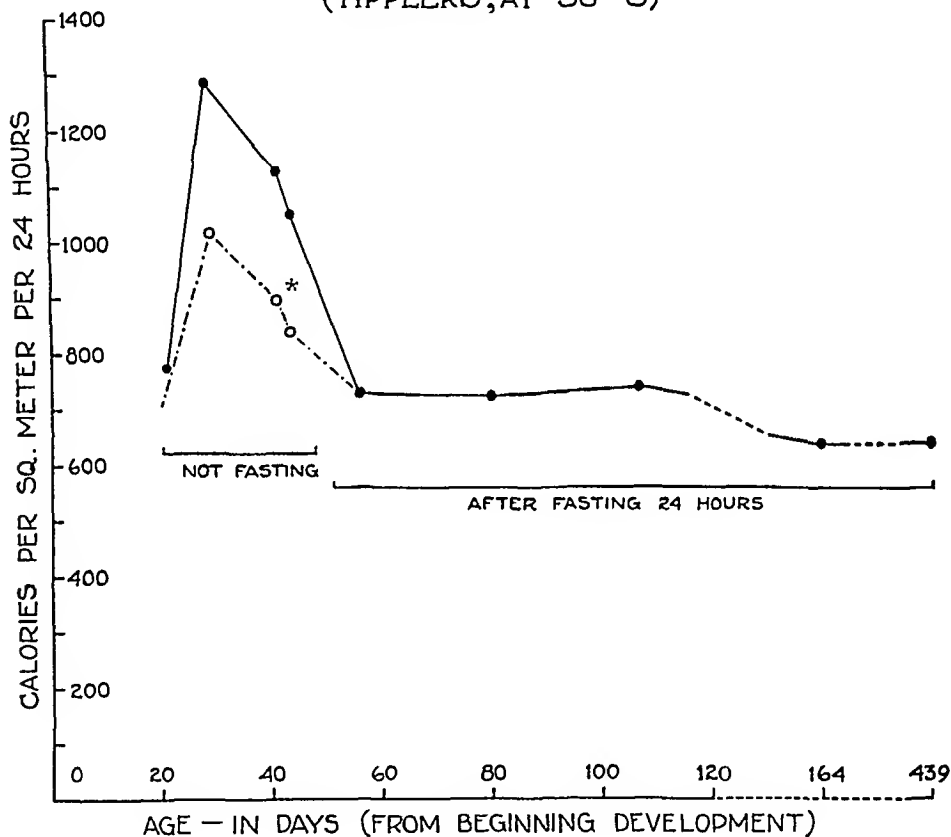


Fig. 1

given in table 1 and in figure 1. The average value obtained from these three fasting birds was 922 calories (separately represented by a * on the curve); their own non-fasting value was 1160 calories. The latter value is therefore 125.8 per cent of the fasting or basal value. If we may suppose that the measured non-fasting values for all birds of the 43-day, 41-day and 29-day stages bear a similar relation to their basal values we obtain the points (open circles) connected by the broken line on figure 1. This

broken line is further extended to a point somewhat below the measured value of the 21-day stage for the reason that the birds of that stage, though burning only fat and protein, were certainly digesting and absorbing food and therefore not in a truly "basal" condition. That portion of the curve which is represented by open circles and dotted lines, together with its continuation in the curve obtained from fasting birds of older stages, best presents the "basal" metabolism of the tippler pigeon during growth when measured at all ages at 30°C.

Details of measurements made at the two youngest stages of growth are of special interest and these are given in table 2. The birds taken for a particular growth stage were not of precisely uniform age, and they varied markedly in weight; an inspection of these differences will supply further evidence for the validity of the first and most irregular part of the growth curve presented in figure 1. Within each age-group it will be noted that smaller size and younger age tend to be associated with a lower metabolism; greater body weight and more advanced age—up to 30 or 32 days—tend to have the higher metabolism. In this connection it should be noted that within very wide limits the actual rate of growth in all pigeons and doves depends upon the volume of the food supply; and that where there is but one young in a nest its growth, during the first 10 days after hatching, may nearly equal that of two squabs in the same nest. Thus no. 24 of table 2 was the sole occupant of a nest; its weight (189 g.) at 29 days (11 days after hatching) was fully twice that of no. 4 (92 g.)—a squab of the same age forced to share with a nestmate the food supplied by its parents—and the metabolism of the former and rapidly growing bird exceeds that of the latter by 20 per cent. Many similar instances of this relationship may be found in this table when one compares two birds of the same age but of very unequal size. We here find a more rapid growth reflected in a higher metabolism. The birds thus compared are of course quite unequal in size; the metabolism values of this paper are, for these intra-specific comparisons, all calculated in terms of heat per square meter of surface.

The rate of growth, in terms of percentage weight increment at the various ages, should be considered. For this purpose we shall use some data of Riddle, Charles and Cauthen (1932) which includes the weights of 15 tipplers from hatching to 105 days. Those birds were never fasted and so present a fair picture of the normal growth of birds of this race. The weights, in grams, for several stages are: Hatching (17 to 18 days) 11.3; 21 days, 30; 28.7 days, 130; 41 days, 230; 43.5 days, 237 grams. A comparison of these values with those obtained (table 1) from the (non-fasting) birds used in the present metabolism measurements makes it clear that the present studies were made on birds growing at the usual or normal rate; also that the repeated measurement of 10 of these birds was without notable effect on their rate of growth. In the other study of the growth rate in

TABLE 2

Individual measurements made on pairs of young at the 21-day stage—20–22 days after beginning of development

Pairs arranged in order of body weight. Next older stage listed below

PAIR (OR NUMBER)	BODY WEIGHT PER BIRD	AGE	RESPIRATORY QUOTIENT	CALORIES PER SQUARE METER PER 24 HOURS	EXACT CHAMBER TEMPERATURE
	<i>grams</i>	<i>days</i>			<i>°C.</i>
1	13.5	21	0.67	624	29.9
2	21.5	21	0.74	674	29.9
3	23.7	20	0.78	675	30.0
4	24.0	22	0.69	898	30.0
5	27.0	21	0.74	778	30.1
6	27.3	21	0.74	773	29.9
7	28.3	21	0.85	701	29.9
8	30.0	21	0.86	742	30.0
9	32.0	21	0.68	914	30.1
10	33.0	22	0.82	828	30.0
11	37.0	21	0.74	875	29.9
12	43.5	22	0.79	846	30.0
13	44.0	22	0.89	714	29.8
Average....	29.6	21	0.768	772.5	29.96

Individual birds, 10–14 days after hatching (29-day stage)

1	88.5	28	0.96	1,148	30.0
2	89	27	1.00	1,261	30.0
3	91.5	27	0.97	1,142	30.0
4	92	29	0.98	1,066	30.2
5	107.0	30	0.86	951	30.2
6	110.5	29	1.08	1,283	30.1
7	117.5	28	0.84	1,095	30.0
8	119	29	1.10	1,145	30.1
9	119	28	1.06	1,312	29.9
10	121	28	0.92	1,150	30.0
11	122.5	29		1,324	30.0
12	124	27	0.92	1,362	29.9
13	127.5	29	0.87	1,234	29.9
14	130.5	28	1.02	1,410	30.1
15	131.5	29	1.04	1,366	30.0
16	134.5	32	1.03	1,231	30.2
17	135.5	27	0.88	1,451	30.1
18	136	28	0.95	1,266	30.0
19	136.5	28	1.05	1,473	30.0
20	149	30	1.11	1,373	30.1
21	159	29	1.09	1,640	30.1
22	162.5	30	1.04	1,408	30.0
23	167	32	1.00	1,435	30.2
24	189	29	1.03	1,285	30.2
Average....	127.5	28.75	0.991	1,283.8	30.05

tipplers weighings were made at 3-day intervals and this permits the following statement on the approximate percentages of growth increment per day at the several stages concerned in the present study: At 21 days, 38 per cent; at 29 days, 14 per cent; at 41 days, 2 per cent; at 43.5 days, 1 per cent. After 45 to 50 days the young are no longer fed by their parents, and our practice is to remove the young when 50 days old. For a few days thereafter they lose weight slightly, and this short period is followed by very slow growth during a few succeeding months. The preceding statement will indicate that, with the important exception of the earliest 21-day stage, the metabolism curve essentially parallels a curve which we might construct for growth increment in this race of pigeons.

Since the present study is especially concerned with extremely early life-stages, and the metabolism of the youngest stage examined bears a peculiar relation to all values obtained later, it is particularly important to consider the adequacy and meaning of the measurements made on our birds 3 days after hatching. The details supplied in table 2 leave no doubt that the value obtained for this stage is approximately correct. These extremely young birds supplied evidence that they already have some capacity to maintain their body temperature considerably above that of their environment. The rectal temperatures of 4 such birds, after remaining for 6-8 hours at 30° in a chamber ventilated at the rate of approximately 2 liters per minute, had body temperatures ranging from 39.4 to 40.0°C.; another group of 4 similarly kept at 36° showed a range of 40.0 to 41.3°. Nine birds of this same age, while covered in the nest by a parent, had an average temperature of 39.2°C. Birds of this stage, given such protection from heat loss as was supplied to these birds, are therefore almost or quite capable of maintaining their normal body temperature when the temperature of the environment is 10°C. lower than their own. These data nevertheless show that when birds of this stage are long held in the chamber at 30° they have a body temperature nearly 2°C. lower than that of the adults, and that their relatively low metabolism was obtained from tissues at a temperature distinctly lower than in all subsequent measurements. When kept in an environment held constantly at 36° these birds seem, however, to maintain body temperature almost identical with that of the adult. Only 8 days later (29-day stage) the average body temperature (night) of the 24 birds studied (at 30°) was 40.4° (1 at 35° was 41.5°), while that of the adults was very slightly less than 41°C.

It can not be assumed that an environmental temperature of 30° is of the same significance to thermolysis in a bird 3 or 11 days after hatching as in the warm-blooded adult pigeon. The "critical" temperature for the adult is approximately 30°C.; but, as suggested by Pembrey's studies, it does not follow that a truly comparable metabolism of unfeathered, partially cold-blooded young of 3 or 11 days is to be obtained at this temperature. Four

measurements (not tabulated) were made in order to learn the relation between the values obtained at 30° and values obtainable at still higher temperatures (35° or 36°C.) on these particular stages. A pair of 20-day young (weight, 14 and 27 g.) and another pair of 20.5-day young (16 and 21.5 g.), at 36°, produced 745 and 710 calories per square meter per 24 hours; from a pair of 25-day young (65 and 80 g.), at 36°, we obtained 1137 calories, and from a 30-day young (107 g.), measured at 35°, 1330 calories. The values obtained from the age-groups thus tested invariably stand in the same relation to each other as when measurements are made at 30°; the heat production at the 25-day and 30-day stages is somewhat increased at the higher temperature; in the 20-day birds, when age and size are fully considered, the metabolism is perhaps 8 per cent higher than that to be expected at 30°. The general form of the curve constructed from 30° values is not changed when the partially cold-blooded stages are measured at a higher environmental temperature at which their cell temperature is made equivalent to that of the adult; at this higher temperature the youngest stage (20-day) again shows a low metabolism, and the 29-day stage again has the highest metabolism of the life cycle.

That a pigeon, while largely (21-day stage) or partially (29-day stage) a cold-blooded animal, should duplicate both the initial low and the succeeding high metabolism found in the warm-blooded young chick is of much interest. That the two cases in birds already find a complete parallel in the three mammals (human, pig, calf) hitherto studied makes it evident that something very important in the physiology of development is involved. The fact that the relatively low initial metabolism occurs in the pigeon when the animal is in phenomenally rapid growth, and the further fact that the maximum metabolism occurs when growth rate (though very rapid) has passed its maximum, demonstrate the extreme complexity of the problem. We do not undertake a discussion of this problem here; we emphasize the now evident fact that for these two phases of metabolic change during early life there is at present no adequate explanation.

SUMMARY

The total metabolism, at 30°C., of a race of common pigeons (tipplers) has been measured at four early stages of rapid growth; basal values were obtained on one of those early stages, at three later periods of slower growth, at early and late adolescence, and after full sexual maturity. The results are based upon 130 measurements.

Three days after hatching (21-day stage), when non-fasting but burning fat only, the bird is in a stage of most rapid growth (38 per cent per day) but it then has a total metabolism only 20 per cent higher than the basal value of the young adult. Eight days later the total non-fasting metabolism (R.Q. of 0.99) is about 100 per cent higher (1284 calories per square

meter per 24 hours) than the basal value at late adolescence (632 calories). This extraordinarily high metabolism coincides with a period of very rapid growth (14 per cent per day) in this animal.

When 41 and 43 days old (23 and 25 days after hatching) the total metabolism and the rate of growth are in rapid decline. At this time measurements made on both fasting and non-fasting birds show that the metabolism is markedly greater (922 calories) than in birds of the 56-day (727 calories) and still older stages.

In general, the basal heat production progressively declines from the 43-day—and probably from the 28-day stage—into adult life; from the twenty-eighth day (10 days after hatching) the decline in metabolism parallels a decline in growth rate.

The curve describing the changes of metabolism with age in the pigeon—an animal which is partially cold-blooded at an early and significant period—is strikingly similar to that already known for man, pig, calf, and fowl. A low metabolism immediately after birth or hatching, this soon followed by the highest metabolism of the life cycle, and the invariable occurrence of both of these things in all of the five species hitherto studied, are challenging facts for which no adequate explanation is now at hand.

BIBLIOGRAPHY

- BENEDICT, F. G. 1919. Boston Med. and Surg. Journ., clxxxi, 107.
BENEDICT, F. G. AND F. B. TALBOT. 1921. Carnegie Inst. Wash. Publ. 302.
BENEDICT, F. G. AND O. RIDDLE. 1929. Journ. Nutr., i, 475, 497.
BRODY, S. AND A. C. RAGSDALE. 1930. Res. Bull., 143, Univ. of Missouri.
DEIGHTON, T. 1924. Proc. Roy. Soc. B, (London), xcv, 340.
DuBOIS, E. F. 1916. Journ. Med. Sci. (N.S.), cli, 781.
GROEBBELS, F. 1927. Pflüger's Arch., ccxviii, 98.
LEICHTENTRITT, B. 1919. Zeitschr. f. Biol., lxix, 545.
MITCHELL, H. H., L. E. CARD AND W. T. HAINES. 1927. Journ. Agric. Res., xxxiv, 945.
PEMBREY, M. S. 1897. Journ. Physiol., xviii, 363.
PLAUT, R. 1921. Zeitschr. f. Biol., lxxiii, 141.
RIDDLE, O., D. R. CHARLES AND G. E. CAUTHEN. 1932. Proc. Soc. Biol. and Med., xxix, No. 9 (June).
WOOD, T. B. 1926. Journ. Agric. Sci. (England), xvi, 425.

THE BASAL METABOLISM OF THE MOURNING DOVE AND SOME OF ITS HYBRIDS

OSCAR RIDDLE, GUINEVERE C. SMITH AND FRANCIS G. BENEDICT

From the Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and the Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.

Received for publication April 2, 1932

The mourning dove (*Zenaidura macroura*) is the only truly feral species of Columbæ (pigeons) which is native to New York. Here it is found in considerable numbers during spring and summer. Nearly but not quite all non-captive individuals migrate southward, to borders of the Gulf of Mexico, in autumn; they return to this locality in April. It is a truly migratory species—avoiding cold weather—and is thus in sharp contrast with the common pigeons and ring doves which we are studying intensively. In these latter species we are observing distinct seasonal differences in the basal metabolism, and also differences in the extent to which the environmental temperature influences the metabolism at the various seasons. The migratory mourning dove, when held captive and measured at various temperatures at various seasons, should therefore provide information of special interest. We have also sought to learn something of the extent to which age, sex and hybridity modify the metabolism in this feral species.

Our stock of birds was formed from a few individuals captured as adults or young near the laboratory; the offspring of these birds are the individuals used in this study. During 3 years we have made 150 measurements upon 39 different individuals. On 15 hybrids 30 measurements were made. The hybrids to be considered here are those derived from mating the mourning dove with a not very dissimilar species from South America (*Zenaida vinaceo-rufa*). The two parent forms are at present classed in different genera, but their full fertility without marked disturbance of the sex ratio may be taken as evidence that they might well be placed in the same genus. The *Zenaidura-Zenaida* hybrids, hereafter to be called Z-Zn hybrids, are fertile, and most of the individuals used in these tests were derived from generations later than F₁. Curiously enough the pure *Zenaida*, a tropical species, is able to produce fertile eggs during all months of the year when given the protection from extreme cold which our glass colony houses afford.

All measurements were made under the standard conditions for pigeons

as earlier described by Benedict and Riddle (1929). These include: Previous fasting for 24 hours (starting this fast, in these small doves, with 5 grams of food plus 5 cc. of water in the crop) during which time the birds are kept in a large glass cage at constant temperature (and the *same* temperature as that to be used in the metabolism measurement); making all measurements at night, with kymograph records of activity, and in completely darkened chambers. Measurements were never started until at least one hour after placing the birds in the darkened chambers, and then only if quiet had been obtained. Closely corresponding values derived from two periods of complete or essential quiet constitute a measurement. Sick or unhealthy birds are excluded. The R.Q. was obtained in 74 of the 150 measurements on mourning doves; its range was 0.67 to 0.76 and its average, 0.718. Similarly, 11 quotients on the Z-Zn hybrids average 0.703. The length of the fasting period (always 24 hours) used with these birds was therefore entirely adequate to obtain fat quotients; perhaps such quotients could have been obtained 4 or 5 hours earlier. In that case a slightly higher metabolism would have been obtained, and the differences we have found between the metabolism of this dove and other domesticated doves and pigeons would be slightly increased.

CONSIDERATION OF DATA. The data for the different seasons, temperatures, sexes, ages and types of birds are summarized in table 1.

Season. In this region non-captive mourning doves lay and fertilize eggs only from April to the end of July; captive birds may begin breeding slightly earlier and continue to the end of August. Soon after the end of the breeding season, but persisting only through the autumn in captive birds, the testes of this species undergo a seasonal involution which is so extensive as to suggest effective functional castration. The time and extent of this involution of the testes are indicated by the following weights of testes of adult doves (both captive and wild) at various months: The average for 6 in May was 514 mgm.; of 5 in August, 435 mgm.; of 4 in September, 87 mgm.; of 2 in October, 18 mgm.; of 5 in December, 396 mgm. Taber (1928) measured the food consumption of this species during the breeding season and in September, and drew the conclusion that "it seems probable that there is a seasonal variation, because the daily food consumption of 13 mourning doves trapped from April 2 to August 1 averaged 11.7 per cent of their body weights, while the daily food consumption of the 9 trapped from September 6 to October 6 averaged 20.4 per cent."

Though the ovary becomes smaller and ovulation ceases before September this gland does not suffer a diminution comparable with that observed in the testis. In the Z-Zn hybrids the gonads are reduced in winter, but much less so than in the pure species and eggs are usually produced in all months except October-January. How then is the basal metabolism related to these seasonal differences in these animals? In our survey of this

TABLE 1

Summary of data on basal metabolism of mourning doves (top) and hybrids

AGE CLASS	SEASON	CHAMBER TEMPERA- TURE	SEX	AGE	BODY WEIGHT	NUMBER OF TESTS	CALORIES PER KILO PER 24 HOURS
		°C.		months	grams		
Immature	Winter	30	♂	9.1	119	5	131.9
			♀	7.1	137	3	119.0
Mature	Breeding	30	♂	26.6	122	8	123.1
			♀	27.8	114	7	117.5
Mature	December	22	♂	46.3	142	6	185.1
			♀	33.9	124	6	165.5
Mature	Breeding	20	♂	27.1	119	19	179.0
			♀	23.8	118	20	175.6
Mature	September	20	♂	34.2	125	5	198.2
			♀	26.1	117	6	195.8
Mature	November	20	♂	29.0	131	10	179.4
			♀	23.8	127	11	168.8
Mature	Winter	20	♂	34.4	137	11	172.3
			♀	29.2	131	11	180.8
Immature	Winter	20	♂	7.4	130	7	172.9
			♀	7.6	116	5	177.6
			?	7.0	115	2	187.3
Immature	Winter	15	♂	7.2	119	5	217.0
			♀	7.1	125	3	200.6

Hybrids—Mourning dove X *Zenaida vinaceo-rufa*

Mature	Winter	22	♂	54.4	108	4	174.6
			♀	39.5	103	4	170.4
Mature	Winter	20	♂	34.6	102	4	192.6
			♀	36.8	105	3	187.4
Mature	Breeding	20	♂	39.4	103	5	184.8
			♀	38.4	98	4	166.4
Immature	Winter	20	?	7.9	102	4	188.5
			♀	6.9	102	2	167.0

point it is necessary to observe that in our work (mostly unpublished) on ring doves and pigeons we find the highest metabolism in the autumn period (September–November), and usually the lowest in summer (June–August), with winter values (December–February) not very much higher than those of summer probably because the colony houses are enclosed and partly heated during the winter (not in autumn).

Our examination of the effects of season on metabolism in the present study is limited essentially to measurements made on mature birds at 20°C. The 39 mourning doves measured (April 28 to August 6) during the breeding season give a mean sex value of 177 calories per kilo per 24 hours, and this is essentially the same as that obtained for November (174) and for winter (177); it is also apparently the same as the December value (175) obtained at 22°, but probably it is 3 to 5 per cent lower than the December value when a derivable temperature correction is made. All these values, however, differ notably from that obtained in September (197) the latter being 11.1 per cent above the summer metabolism. Thus the highest value is found at a time when the testes are being actively reduced, but the very fact that the testes are being resorbed makes it entirely probable that they are then contributing some sex hormone to the body; also, this would seem to be the time when the thyroids should be actively enlarging in response to colder weather—if the thyroids of these birds enlarge in cold weather as do the thyroids of ring doves and common pigeons (Riddle and Fisher, 1925).

A tentative explanation of these unusual and unexpected seasonal values may be offered. It is conceivable that the "breeding season" values reflect two opposed tendencies: First, a tendency to a lowered summer metabolism (as in other doves and pigeons) accompanying reduced thyroid activity; second, a failure of the summer metabolism to become actually lower because of a stimulating action of the gonad hormones—whose presence *in this species* is probably essentially limited to this breeding period. We may likewise suppose that the actual level of the metabolism in November is the resultant of two things—the loss of gonad hormones (tending to lower it), but a gain of thyroid activity (in response to cold) which increases it. However, the fact that the "winter" value is no higher than that for the breeding season, does not well fit this interpretation since, as noted above, the testes have then recovered much of their former size and presumably much of their endocrine function. Again, the high metabolism in September may be partially or wholly a result of less effective feathering coincident with a possible predominance of molting at this period, though we lack evidence that this is the molting period for this species. Even this very questionable disposition of the high metabolism in September would leave wholly unexplained the equivalence of the metabolism in summer, November and winter.

If the explanation suggested above is erroneous or inadequate it would seem necessary to conclude that in this migratory, cold-avoiding species the thyroids do not respond to cold by increased function as they do in non-migratory doves and pigeons. In that case our results suggest the probability of a causal relationship between thyroids which do not make the normal response to cold and the presence of the migratory instinct in birds possessing such thyroids. Special evidence on this point is found in the fact, first observed by Riddle and Fisher (1925), that the thyroids of non-migratory ring doves and common pigeons undergo marked enlargement and increased function during autumn and winter, and by Haecker's (1926) later demonstration of the same thing in the non-migratory European crow.

Environmental temperature. Immature mourning doves were measured in winter at 15°–20°–30°C. Neglecting here a small sex difference (the sex of 2 birds being unknown) the tabulated values indicate that the metabolism at 20° has been reduced by 16.2 per cent below the 15° value; at 30° their metabolism, based on the means for the sexes, is reduced by 29.8 per cent below the value found at 20°. Thus a rise of temperature from 15° to 20° lowers the metabolism by 3.23 per cent per degree, and between 20° and 30° the rise in temperature lowers this metabolism, on the average, by 2.98 per cent per degree. A similar comparison for the mature doves can be made only for 20°–30°, and in the "breeding season." Here the metabolism at 30° indicates a loss of 3.2 per cent per degree from the value found at 20°C.

It should be noted that these percentage changes in the metabolism per unit of change in the environmental temperature seem relatively greater in the mourning dove than those found in ring doves and pigeons. Riddle, Christman and Benedict (1930) found that in ring doves the two sexes gave a different response to temperature change, but that between 20° and 30° an average was 2.61–2.99 per cent in males, and 2.03–2.25 per cent per degree in females; between 15° and 20° this value was 2.33 in males, 2.02 in females. Our own data (1932) on a race of common pigeons (tipplers) show that, though values obtained from calculations of this sort depend essentially upon season, the metabolism change for measurements made on those birds at 30° and 20° lies between 0.3 and 1.0 per cent per degree; between 15° and 20° the extent of the influence upon the metabolism ranges between 1.3 and 3.4 per cent per degree. These values for common pigeons are the mean values for the sexes. Thus almost all of the several available comparisons indicate that the metabolism of mourning doves, though apparently less modified by prolonged seasonal temperature change, is more increased by a temporary lowering of the environmental temperature (for purposes of measurement) than is that of ring doves and common pigeons. In other words, the migratory species studied here must

do more work—must produce relatively more heat—to maintain itself in a cold climate than is required in two somewhat related non-migratory species. Since the mourning dove is only slightly smaller than the ring dove we here again meet a fact that may be intimately, perhaps causally, related to the migrant character of this species.

Age. Measurements made at 20° on mourning doves during the winter, when the gonads of immature and mature birds are relatively inactive and when body size in the immature is nearly equal to that of the adult, indicate that birds 6 to 8 months old have nearly or quite the same metabolism as birds aged 20 to 50 months. This "winter" metabolism of the immature birds is also entirely similar to the metabolism of mature birds in November and in the breeding season, but is definitely lower than that of birds 20 to 50 months old measured in September. The metabolism of eight immature birds measured at 30° in winter seems only slightly higher (4.3 per cent average) than that of fifteen mature birds measured at this temperature during the breeding season. A few measurements made on Z-Zn hybrids 6-9 months old at 20° during winter indicate no superiority of their metabolism over that of either of the two groups of mature birds measured at 20° in winter. These data make it entirely probable that, between 6-50 months, age is a negligible factor in the metabolism of mourning doves and their hybrids.

Sex. Within the several groups studied there is a tendency for the males to be slightly older and larger than the females. Much experience has taught us that the relation of the sex factor to metabolism in species and races of doves and pigeons can not be adequately determined by a few scores of measurements; nor yet by many hundreds of measurements made at a single temperature which may affect the metabolism of the two sexes differentially. In this migratory, cold-avoiding species we note that the metabolism of males is not obviously depressed more than is that of females by measurement at an environmental temperature of 30°C. The present data were obtained at four different environmental temperatures and seem to give significant indications concerning the sex factor in the metabolism of mourning doves, and probably also in the hybrids. At all of the four temperatures used the male mourning doves are indicated as having a basal metabolism higher than that of the females; in 7 of the 9 groups measured the males show the higher value; the means obtained from the 9 group values indicate that the males have a metabolism 3.8 per cent in excess of that of the female when the heat production values are calculated on the basis of body weight, and when calculated in units of body surface this excess is 4.6 per cent.

Even the fewer data concerning sex in the Z-Zn hybrids are not negligible since, in the 3 groups studied, they afford consistent evidence that the metabolism of the males at 20° to 22° is higher than that of the females.

The means derived from the three available groups indicate that the males have a metabolism 5.3 per cent higher on the basis of weight, and 6.3 per cent higher per unit of body surface. These data have an added interest from the fact that they seem to be the first specific (or generic) hybrids upon which measurements of the respiratory metabolism have been made.

Species. The metabolism of the mourning dove may be compared with that of ring doves. For mature individuals of 16 races of the latter species, measured at all seasons at 20°C. (a few at 22°), a mean value of 155 (males) and 150 (females) calories per kilo per 24 hours (811 and 774 per square meter per 24 hours) was found by Riddle, Christman and Benedict (1930). These values are somewhat lower than those here obtained for the mourning doves at 20° to 22° (table 1). Five groups (all seasons) of mature mourning doves give mean values of 183 and 177 calories per kilo per 24 hours, or 923 and 885 per square meter per 24 hours, in males and females respectively. Some wholly comparable data for a small race of common pigeons (tipplers) gave 61 males a value of 105 calories per kilo per 24 hours, and 673 calories per square meter per 24 hours; from 70 females we obtained 108 calories per kilo per 24 hours, and 687 per square meter per 24 hours. Other races of normal common pigeons do not greatly exceed this value. It is thus shown that the feral species has a metabolism higher than that of domesticated doves and pigeons. We have evidence that the true normal rectal temperature of the mourning dove is no higher than that of ring doves or common pigeons. The metabolism of the wild or Norway rat was likewise shown by Benedict and Petrik (1930) to exceed that of the domesticated albino rat.

The Z-Zn hybrids have a body weight about 20 per cent less than that of mourning doves and probably only about equal to that of the pure *Zenaida* ancestry—but unfortunately only a single female of the latter species was available for study. The metabolism of the hybrids is equivalent to that of the mourning doves when calculated on the basis of weight (184 and 175 calories in males and females), but about 7 per cent less when calculated on a basis of surface area (871 and 813 per square meter per 24 hours). Estimates of surface area in these several calculations assume that the area varies in proportion to the two-thirds power of the body weight; $K = 10.0$.

SUMMARY

Individuals of a feral, cold-avoiding, migratory species of dove, when reared in captivity, have been found to have a higher basal metabolism, measured at 15°, 20° and 30°C., than that found in related non-migratory domesticated doves and pigeons. At 20°, and measured at all seasons, the mean values found for the two sexes in these several species are: 904

(mourning dove), 792 (ring dove), and 680 (tippler pigeon) calories per square meter per 24 hours.

Seasonal changes in the metabolism of this species are especially difficult to interpret because possible responses to hot and cold weather and an extraordinary autumnal involution of the gonads, apparently the equivalent of functional castration, are involved. The highest metabolism is found in September; lower and quite interchangeable values are found during the breeding season, November and winter.

It is possible that the thyroids of this species do not respond to cold weather by increased activity as do those of non-migratory forms and that this is significantly or causally related to the migratory instinct.

This migratory, cold-avoiding species must do more work—must produce relatively more heat—to maintain itself at lower temperatures than is required in either of two related non-migratory species of dove already studied.

Age is here a negligible factor in the rate of heat production of birds whose ages range between 6 and 50 months.

The metabolism of male mourning doves, as measured at all temperatures and during all seasons, was higher (173 per kilo, and 873 calories per square meter, per 24 hours) than that of females (167 and 829 calories). Per unit of weight this excess is 3.8 per cent, and per unit of surface it is 4.6 per cent.

Thirty measurements made at 20° and 22° on hybrids between the mourning dove and the Zenaida dove indicate a metabolism (179 calories per kilo per 24 hours) that is nearly the same as was found in the mourning dove (180 calories), that is equal in younger and older birds, and that is higher by 5.3 per cent (weight) or 6.3 per cent (surface) in males than in females.

BIBLIOGRAPHY

- BENEDICT, F. G. AND O. RIDDLE. 1929. *Journ. Nutr.*, i, 475, 497.
BENEDICT, F. G. AND J. M. PETRIK. 1930. *This Journal*, xciv, 662.
HAECKER, V. 1926. *Schweiz. med. Wochenschr.*, no. 15, 337.
RIDDLE, O. AND W. S. FISHER. 1925. *This Journal*, lxxii, 464.
RIDDLE, O., G. CHRISTMAN AND F. G. BENEDICT. 1930. *This Journal*, xciv, 111.
RIDDLE, O., G. C. SMITH AND F. G. BENEDICT. 1932. (In press).
TABER, W. B. 1928. *Auk.*, xlv, 339.

THE PHYSIOLOGIC ACTION OF DEHYDROCHOLIC ACID

J. F. REGAN AND O. H. HORRALL

From the Physiological Laboratory of the University of Chicago

Received for publication April 4, 1932

Dehydrocholic acid, according to Neubauer, caused a two- to fivefold increase in bile secretion on intravenous injection in rabbits and in patients with biliary fistulae. On the basis of other work on cholic and desoxycholic acid, he stated that the cholagogic bile acids increase the bile secretion pressure.

Neubauer reported further that dehydrocholic acid is less hemolytic (human, rabbit erythrocytes) than desoxycholic acid, that it is $\frac{1}{25}$ as toxic to the excised frog heart as desoxycholic acid, and that it has only about $\frac{1}{8}$ the toxicity of desoxycholic acid for guinea pigs. This was confirmed by Gillert.

Neubauer injected into one dog weighing 8.8 kilograms on different days sodium dehydrocholate in the following amounts: 0.6, 1.55, 3.2, 3.8, grams, that is, from 0.068 to 0.43 gram per kilogram of body weight, in from 6 to 32 per cent solutions, without toxic effects. On injection of 0.51 gram per kilogram intravenously vomiting and diarrhea were observed. The intravenous injection of 2 grams of sodium dehydrocholate in 20 per cent solution in patients was without toxic effects.

In beginning our work solutions of sodium dehydrocholate¹ and sodium choleate were injected into frogs (*Rana pipiens*) weighing from 40 to 60 grams. Some injections were made into the anterior lymph sac and some into the posterior lymph sac. The salts were prepared by dissolving the acids in warm alcohol and neutralizing with N/10 sodium hydroxide, using phenolphthalein as the indicator. The solutions were then evaporated to dryness and the salt taken up in distilled water.

In tests on 36 frogs with sodium dehydrocholate (0.2 to 5.0 millimols per kilogram) and 14 frogs with sodium choleate, the sodium dehydrocholate proved to be about $\frac{1}{8}$ as toxic as the latter salt.

On intravenous injection of 20 per cent solution of sodium dehydrocholate in seven dogs, defecation, vomiting, hyperexcitability and death were observed following doses ranging from 0.024 to 0.72 gram per kilogram body weight. Similar reactions were produced by sodium glyco-

¹ The dehydrocholic acid (Decholin) used in all these experiments was obtained from the Riedel-de Haen Company.

cholate in smaller doses. Hypodermic injections of sodium dehydrocholate in concentrations up to 20 per cent do not induce local necrosis as does sodium glycocholate.

In all the following experiments the dogs were under barbitol anesthesia—300 mgm. per kilogram per os. In all experiments whether determining bile flow or bile pressure the common bile duct was cannulated and the cystic duct ligated. All injections were made into the femoral vein. The dose was arbitrarily taken as 10 cc. of 20 per cent solution or 2 grams since it is the calculated human dose. The solutions were always warmed

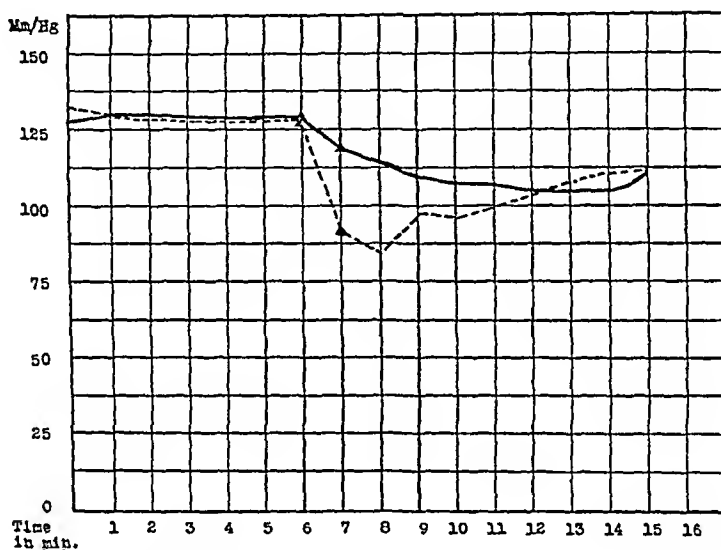


Fig. 1. The effects of intravenous injection of sodium dehydrocholate and sodium glycocholate on blood pressure in barbitolized dogs.

———— Average blood pressure of 18 dogs following injection of 2 grams of sodium dehydrocholate.

----- Average blood pressure of 11 dogs following injection of 2 grams of sodium glycocholate.

▲—▲ Injection.

to body temperature and the injections made at a constant rate in about one minute.

Figure 1 gives the average fall in blood pressure in a large number of dogs following the intravenous injection of 2 grams of sodium dehydrocholate and the same dose of sodium glycocholate (Merck). The limits of fall were from 4 to 80 mm. of mercury for sodium dehydrocholate and from 24 to 104 mm. of sodium glycocholate. It is readily seen that sodium dehydrocholate has less effect on blood pressure than sodium glycocholate.

There was no change in the average respiratory rate of 19 dogs following injection of 2 grams of sodium dehydrocholate. However, nine dogs

showed an increase in rate and ten dogs a decrease. With similar amounts of sodium glycocholate there was an average decrease in respiratory rate of 18.70 per cent. Eight dogs showed a decrease whereas three dogs showed no change.

TABLE 1
Amounts of bile secured in 15 minute intervals

DOG	AVERAGE BASAL	AVERAGE FOR 2½ HRS. AFTER INJECTION OF Na DEHYDROCHOLATE
	cc.	cc.
344	0.85	7.21
345	0.44	6.38
346	0.76	4.96
347	1.30	6.30
348	0.46	4.30
350	0.69	6.05
351	0.33	6.26

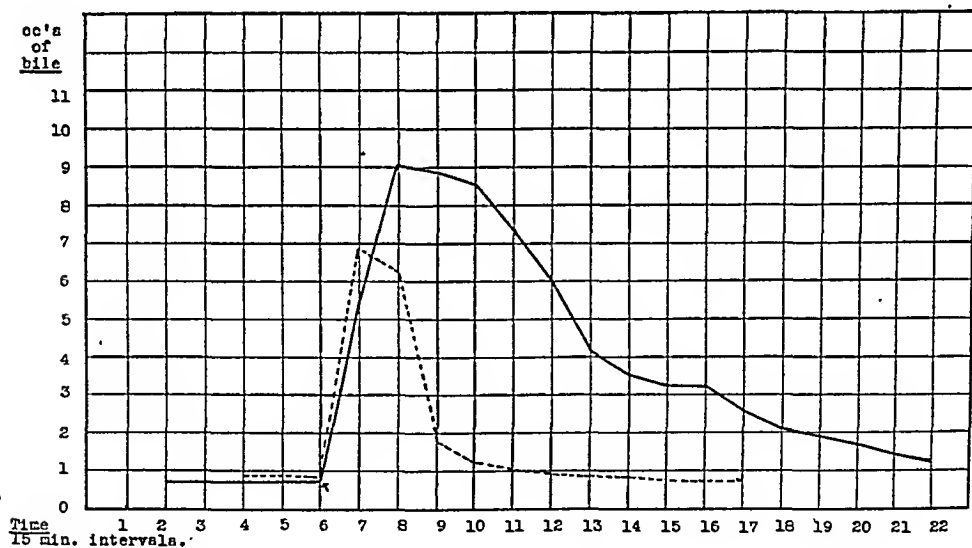


Fig. 2. Bile production following injection of bile salts.

↑ = Injection.

———— Bile production following intravenous injection of 2 grams of sodium dehydrocholate. Average for seven dogs.

----- Bile production following intravenous injection of 2 grams of sodium glycocholate. Average for two dogs.

Bile was collected at fifteen minute intervals by means of a cannula in the common duct, the cystic duct being ligated. Control periods were run for at least one hour. Sodium dehydrocholate was then injected.

As shown in table 1 there was an increase in bile flow from 4.8 to 19 times the basal, the average being about 10 for $2\frac{1}{2}$ hours following the injection in

TABLE 2
The effects of bile acids on total solids

DOG NUMBER	GRAMS OF SOLIDS PER 100 CC. OF BILE BEFORE Na DEHYDROCHOLATE	GRAMS OF SOLIDS PER 100 CC. OF BILE AFTER Na DEHYDROCHOLATE	PERCENTAGE CHANGE IN TOTAL SOLIDS
344	8.15	4.51	-48.57
	8.05	3.91	
	7.83	4.06	
	Av. 8.01	Av. 4.12	
345	7.91	3.77	-55.07
	8.88	3.74	
		3.80	
	Av. 8.39	Av. 3.77	
346	7.17	3.35	-59.60
	7.17	3.12	
	7.40	2.94	
		2.63	
		2.62	
	Av. 7.25	Av. 2.93	
347	7.94	4.09	-49.13
	6.56	4.21	
		3.67	
		3.52	
		2.97	
	Av. 7.25	Av. 3.69	
348	11.98	3.90	-68.66
	9.84	3.47	
		2.88	
	Av. 10.91	Av. 3.42	
350	7.70	4.02	-53.46
	7.64	4.10	
		2.60	
	Av. 7.67	Av. 3.57	
351	11.04	4.08	-65.31
		3.96	
		3.45	
		Av. 3.83	

seven dogs. The bile began to flow faster within one minute after injection. The length of time required for the bile flow to return to normal was from 2 to 5 hours, the average being $3\frac{1}{2}$ hours.

Figure 2 is a graph of the bile flow showing the average for seven dogs. In all experiments with exception of one the bile changed from a dark greenish brown to a clear amber color. The one changed to a clear red color—resembling hemolyzed blood.

Total solid determinations were run on these samples (see table 2). There was a marked decrease in solids, more marked in the later samples.

Bile was collected at 15 minute intervals from two dogs. After obtaining the basal flow for over an hour we injected into one, a female weighing 20 kilograms, 10 cc. of a 20 per cent solution of sodium dehydrocholate, and into the other, a male weighing 26 kilograms, 10 cc. of a 20 per cent solution of sodium glycocholate (Gorlitz). In both cases there was an increase in the amount of bile produced per 15 minute interval. When bile flow had returned to nearly basal we injected sodium glycocholate in the dog

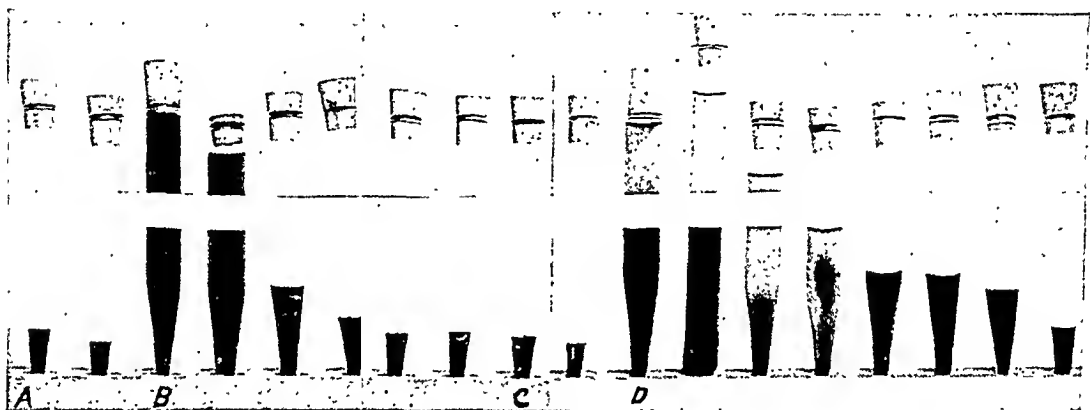


Fig. 3. Amounts of bile collected per 15 minute interval.

A, basal bile flow; B, bile flow following the intravenous injection of 2 grams of sodium glycocholate; C, basal bile flow; D, bile flow following the intravenous injection of 2 grams of sodium dehydrocholate.

which had previously received sodium dehydrocholate, and vice versa. There was an immediate increase in bile flow. In both dogs, as may be readily seen in the photographs of the tubes containing the bile collected per 15 minute interval, the sodium dehydrocholate caused a greater flow of bile and had a more prolonged action than the sodium glycocholate.

Bile pressure determinations were made on seven dogs by connecting a cannula in the common duct with a bromoform manometer. The cystic duct was ligated in each case. The bile was allowed to reach its maximum secretion pressure and then 10 cc. of 20 per cent sodium dehydrocholate injected intravenously. The average normal bile pressure was found to be 341 mm. of water. As a result of the injections the average pressure rose to 385 mm. of water or a 13 per cent increase.

Hemolysis experiments were run on sodium dehydrocholate according

to the method of Ponder, using human corpuscles. We found that it, in comparison with some very pure sodium glycocholate in similar concentrations, has very slight hemolytic effect.

Though all our data tend to show that dehydrocholic acid is much less toxic than glycocholic acid we are of the opinion that it does produce some toxic effects in dogs, in the doses which we have used. We have shown that it produces toxic effects even when such small quantities as 0.024 gram per kilogram are given. Neubauer concluded from his work on one dog that it was non-toxic in quantities up to 0.4 gram per kilogram when given intravenously. He does not give the rate of injection. The amounts of dehydrocholic acid used in these experiments were relatively much greater than that advised for the human.

SUMMARY

1. Dehydrocholic acid is not as toxic for frogs as cholic acid.
2. Dehydrocholic acid has toxic effects on dogs when given intravenously—but not as marked as those produced by glycocholic acid.
3. Dehydrocholic acid causes a fall in blood pressure, more prolonged but not as great as that produced by similar quantities of glycocholic acid.
4. Dehydrocholic acid causes a marked increase in bile flow—greater and more sustained than that produced by glycocholic acid. Bile secretion pressure is also increased.
5. Dehydrocholic acid has little effect on respiration.
6. Dehydrocholic acid causes an absolute increase but a relative or percentage decrease in total solids in the bile.
7. Dehydrocholic acid is hemolytic for red corpuscles only in high concentrations.

The writers are indebted to Dr. A. J. Carlson, under whose direction this work was conducted.

BIBLIOGRAPHY

- GILLERT, E. 1926. *Zeitschr. f. d. ges. exper. Med.*, lii, 779.
NEUBAUER, E. 1922. *Biochem. Zeitschr.*, cxxx, 556.
1923. *Klin. Wochenschr.*, ii, 1065.
PONDER, E. 1921. *Proc. Roy. Soc. Biol.*, xcii, 285.

DEMONSTRATION OF THE ACCELERATOR NERVE AND OF POSTGANGLIONIC PARASYMPATHETIC FIBERS IN THE VAGO-SYMPATHETIC TRUNK OF THE DOG

LAWRENCE O. MORGAN AND PHILIP P. GOLAND

From The Department of Anatomy, University of Cincinnati College of Medicine

Received for publication April 9, 1932

During the latter part of the nineteenth century several investigators described an accelerator nerve in the vago-sympathetic trunk in mammals (Schmiedeberg, 1871; Boehm, 1875; Schiff, 1878; Bayliss, 1920; Arloing, 1896, and others). Later investigations on the cardio-accelerator action of the mammalian vagus have given conflicting results.

Tulgan (1923) found that when the vagus endings in the heart were paralyzed in the cat by the use of atropine, there remained in the vagus nerve fibers which cause an acceleration of the heart rate. Dale, Laidlaw and Symone (1910) also demonstrated an accelerator action in the vagus of the cat. They found that acceleration of the heart could still be produced by stimulating the vagus after the cervical sympathetics had been removed. These investigators conclude that the reaction is due either to a reversal of function of normally inhibitory fibers or to the presence of masked accelerator fibers belonging to the vagus. Stewart (1918) believes that the vago-sympathetic trunk in the dog usually carries some cardio-accelerator fibers. Reed and Layman (1930) found that stimulation of the peripheral end of the cut vagus in the dog with a weak tetanizing current sometimes caused an acceleration of the heart and a rise in blood pressure while a stronger current gave the typical vagus response.

Gaskell (1920) states that without exception, the cardio-motor fibers arise from the thoracico-lumbar divisions of the autonomic nervous system. After extensive investigations on the subject Hering (1924) concludes that the vagus or sympathetic trunk in the neck contains no accelerator fibers in the dog, cat, rabbit or monkey, although he admits that a weak stimuli to the intact vagus (especially on the right side) may accelerate the heart. Hering (1914) believes this to be due to a reflex action.

Two investigators have suggested the presence in the vagus of still another mechanism which probably represents postganglionic para-sympathetic neurones having their cell bodies in the nodose ganglia. According to Winkler (1918) the nodose ganglion is to be considered as the autonomic vertebral ganglion. It is here that the preganglionic course of the

autonomic fibers of the vagus and accessory nerves provisionally terminate. Koreiša (1931) found that after the vagus had been sectioned intracranially, and the dorsal nuclei allowed to degenerate, there remained vagus fibers beginning in the nodose ganglion which carry inhibitory impulses to the heart.

It is the purpose of the authors to present additional evidence concerning the presence of the accelerator nerve in the vagus of the dog. We have also obtained considerable evidence in support of the view that there are sometimes present in the vago-sympathetic trunk postganglionic fibers (especially on the left side) which, when stimulated, cause a fall in blood pressure and sometimes a slowing of the heart.

MATERIAL AND METHODS. As far as possible, only healthy young dogs were used for this investigation. In 25 animals the operation was successfully performed and adequate records obtained. Of this number 14 were used for a study of the left vagus and 11 were used for a study of the right vagus. First the animal was anesthetized and either the right or left vagus nerve cut proximal to the nodose ganglion as close as possible to the point where the vagus emerges from the jugular foramen. In three cases a small arterial clamp was placed on the nerve in this position instead of the nerve being severed. With two exceptions the animal was allowed to live for a period of 11 to 14 days. This period was calculated to allow ample time for the cut fibers to degenerate sufficiently so that they would not respond to electrical stimulation. As a check, two animals were allowed to live for only 8 days and it was found that the vagus did not respond to stimulation at the end of this shorter period.

Some difficulty was experienced in controlling hemorrhage due to blood vessels which run within the sheath of the vagus. It was found that this hemorrhage could be avoided by placing a small hemostat on the vagus at its point of emergence from the jugular foramen and cutting the nerve distal to the hemostat. The hemostat was then allowed to remain in position for a few minutes after the nerve was cut. Care was exercised in exposing the nerve only at the point at which it was to be cut and in avoiding injury as far as possible to the nodose ganglion.

Eleven to fourteen days after the operation the dog was placed under ether anesthesia and prepared for a study of the functional activity of the vagus which had previously been cut. A cannula was placed in the carotid artery on the unoperated side and connected to a mercury manometer arranged to record the heart rate and blood pressure on a smoked drum. The respiration was also recorded by means of a tracheal cannula connected to a respiratory tambour. A signal magnet was arranged to write on the smoked drum and connected in the primary circuit with an induction coil. The strength of the current was occasionally varied during the experiments but a tetanizing current of moderate strength was usually found

to be most effective in producing a response in the vagus. The vagi nerves were then exposed in the neck region and a series of records taken while either the normal or cut nerve was being stimulated. After the intact nerve had been stimulated the nerve was then cut in most cases and the peripheral and central ends stimulated.

A post-mortem examination was made to determine if the vagus had been completely severed proximal to the nodose and the nodose ganglion on the operated side was sectioned and stained for histological study.

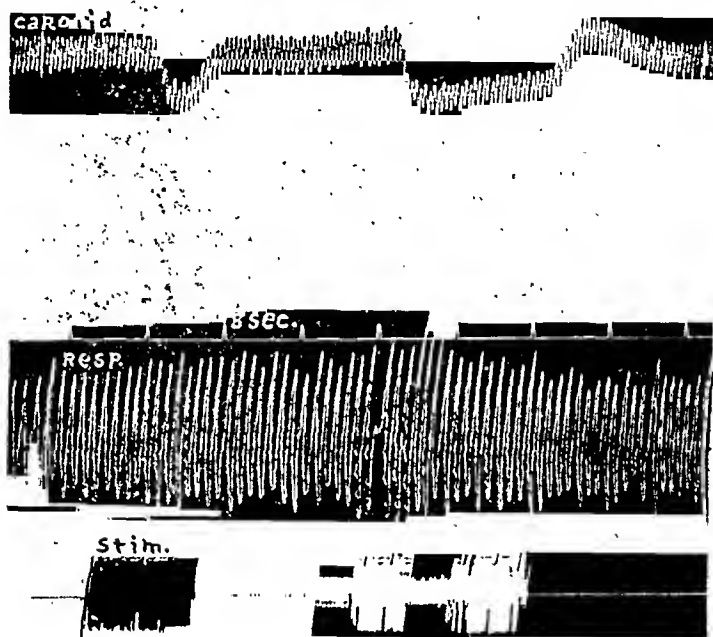


Fig. 1. Case 1. A record of the blood pressure and heart rate during stimulation of the left vago-sympathetic trunk. The left vagus had been cut proximal to the nodose ganglion 13 days previous.

DISCUSSION. After the vagus has been sectioned proximal to the nodose ganglion and the cut fibers allowed to degenerate the changes in heart rate and blood pressure obtained by stimulating the vago-sympathetic trunk are illustrated in figures 1 to 3.

Figure 1 (case 1, left vagus) illustrates the fall in blood pressure which was usually obtained with the left vagus and occasionally with the right. With the first stimulus of 11 seconds' duration there was a latent period of 8 seconds after which the blood pressure fell from 146 mm. to 122 mm. and remained at that level for the remaining 3 seconds during which the stimulus was applied. With the fall in pressure the heart rate decreased from 127

to 120 beats per minute. With a second stimulus of 22 seconds' duration there was a latent period of 8 seconds; the blood pressure fell from 148 mm. to 122 mm., while the heart rate increased from 127 to 135 beats per minute.

Figure 2 (case 3, left vagus) is a record illustrating the response obtained when the vago-sympathetic trunk was cut in the neck and the peripheral end stimulated. With a stimulus of 20 seconds' duration the blood pressure fell from 120 mm. to 98 mm. The heart rate decreased from 150 to 127 beats per minute.

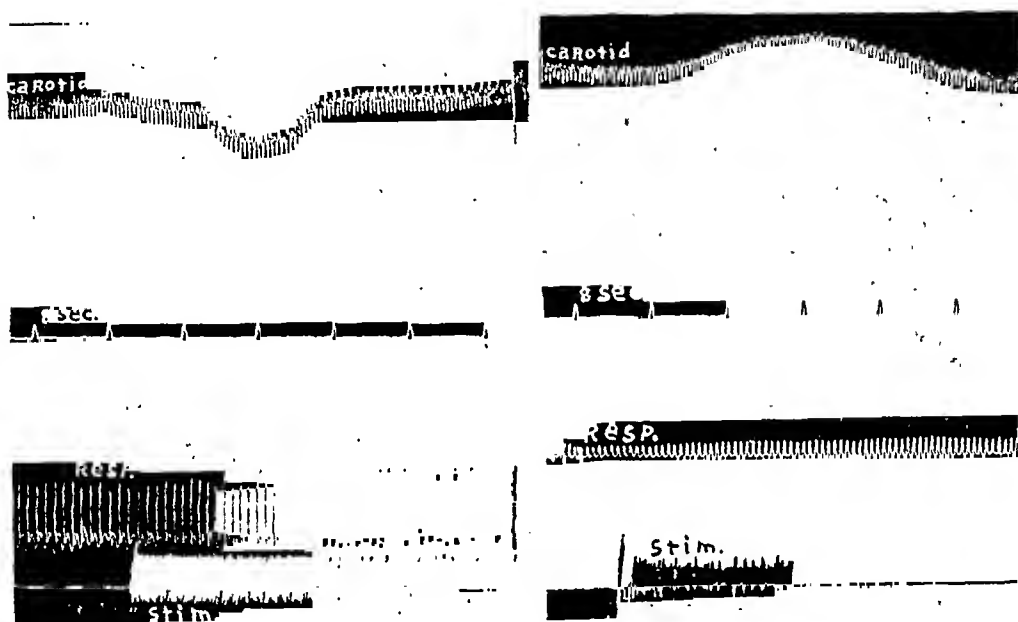


Fig. 2

Fig. 3

Fig. 2. Case 3. A record of the blood pressure and heart rate during stimulation of the peripheral end of the cut vagus on the left side. The vagus had been severed proximal to the nodose ganglion 14 days previous.

Fig. 3. Case 11. A record of the blood pressure and heart rate during stimulation of the left vago-sympathetic trunk. The vagus had been cut proximal to the nodose ganglion 12 days previous.

Figure 3 (case 11, left vagus) illustrates a second type of response frequently obtained on either the right or left side. In this case when the vago-sympathetic trunk was stimulated for a period of 18 seconds, the blood pressure rose from 126 mm. to 140 mm. and the heart rate increased from 138 to 168 beats per minute.

A study of the experiments described above shows that after the vagus is severed from its connection with the medulla, proximal to the nodose

ganglion, there may still remain in the vago-sympathetic trunk two active groups of motor fibers. One of these fiber groups, upon stimulation, causes a fall in blood pressure and frequently a slowing of the heart rate. A second group of fibers resembles the accelerator nerve which has been previously described by other investigators.

Among the 14 animals, in which the left vagus was cut above the nodose ganglion, stimulation of the left vagus caused a fall in blood pressure in 10 cases (cases 1 to 10). In 4 of these cases (cases 2, 4, 5, 7) there was sometimes a rise in blood pressure during stimulation while at other times there was a fall in pressure. In one case (case 11) a rise in blood pressure was the only response to stimulation. In 3 cases (cases 12, 13 and 14) there was no response to stimulation of the left vagus.

Among the 11 animals in which the right vagus was cut above the nodose ganglion, only two (cases 15 and 16) showed a slight fall in blood pressure when the right vagus was stimulated. In these two cases there was sometimes a rise in blood pressure during stimulation. In fact, a rise in blood pressure was more frequently elicited and more pronounced than the fall in pressure which sometimes occurred. In 3 cases (cases 17, 18 and 19) a rise in blood pressure was the only response to stimulation of the right vagus.

From the above summary it will be seen that the neurone which is responsible for lowering blood pressure is apparently present much more frequently on the left side than on the right. On the left side a lowering of blood pressure was obtained in 71 per cent of the cases studied. On the right side a lowering of the pressure only occurred in 18 per cent of the cases. While our series of animals is too small and the variability in the response to stimuli too great to enable us to state that this mechanism is normally present in a definite percentage of cases, these experiments do indicate a difference between the right and left side. The so-called accelerator nerve in the vagus which causes an acceleration of the heart and a rise in blood pressure was demonstrated in almost the same percentage of cases on the right and left sides.

There are two possible factors concerned with the fall in blood pressure which occurs with stimulation of the vagus nerve. The first of these is a slowing of the heart which may result in a decrease in the output of blood from the heart. The second factor which may play a rôle in lowering blood pressure is a dilatation of the blood vessels in the viscera. Although we have no positive proof of the relative importance of these two factors in our operated animals, an examination of the blood pressure tracings may be of interest. In those cases in which the left vagus was cut proximal to the nodose ganglion and the animal allowed to live for 12 to 14 days, the results were as follows:

Of the records which showed a definite lowering of blood pressure when

the left vagus was stimulated, there was a definite slowing of the pulse in six instances. Three of the tracings showed a lowering of pressure with no change in pulse rate, while in three instances the pulse rate was accelerated. In both of those cases in which stimulation of the right vagus caused a fall in blood pressure, there was a marked acceleration of the pulse rate. These observations suggest the probability that dilatation of the blood vessels in the viscera plays a more important rôle in lowering the blood pressure in these cases than does a slowing of the heart rate.

In those cases in which there was a rise in blood pressure following stimulation of the vagus on the operated side, the rise in pressure was accompanied by a marked increase in pulse rate in all but two cases. In cases 17 and 18 there was a rise in blood pressure with no apparent change in heart rate. The accelerator nerve was demonstrated in five cases on the left side and five on the right.

It will be noted that the parasympathetic neurone, which remains intact after the vagus has been severed from the medulla, responds to stimulation in a manner quite different from the normal vagus. While the normal vagus responds very quickly to stimulation and the blood pressure falls suddenly to a low level, the operated vagus responds more slowly, usually after a latent period of a few seconds. In the operated animals it was sometimes necessary to stimulate the nerve for several seconds and to repeat the stimulation a number of times before a response could be elicited. The lowering of the blood pressure usually proceeded gradually, was rather prolonged and then was slow in returning to normal. By means of the potential record Bishop and Heinbecker (1930) have distinguished four types of fibers in the vagus nerve. These fiber groups differ in conduction rates and in such properties as threshold, chronaxie, refractory period, and duration of axon potential response.

A histological and cytological study was made of the nodose ganglia on the side on which the vagus had been previously sectioned, proximal to the ganglia. Only a slight amount of chromotolysis was present among the ganglion cells and that was not confined to any one type of cell. This study gave no definite evidence which could be applied to our problem.

Although our investigation indicates that an accelerator nerve can frequently be demonstrated in the vago-sympathetic trunk of the dog, the origin of this nerve is still unknown. After section of the vagus proximal to the nodose ganglia there remains an intact neurone (especially on the left side) which is capable of lowering blood pressure and slowing the heart. We are probably dealing here with a postganglionic parasympathetic neurone. The cell body of this neurone is probably located in the nodose ganglion. According to Kure, Nitta, Tuzi, Siraisi and Suyenaga (1918) there are postganglionic parasympathetic neurones with their cell bodies located in the dorsal root ganglia of the spinal cord. These authors review

the work of Imagawa, Sunago, Kozima and Sakurazawa which indicates that similar parasympathetic fibers run in the chorda tympani, and that there are postganglionic parasympathetic fibers having their cells of origin in the ciliary and sphenopalatine ganglia. In addition to the five cell types which are present in spinal ganglia, Clark (1926) found 90 per cent of the cells of the nodose ganglia to be of another type (type F) which is not present in the spinal ganglia. A majority of the cells in the jugular, glossopharyngeal and geniculate ganglia were also found to belong to class F. Molhant's (1912) localization of cell groups in the nodose ganglia, according to the regions which they innervate, does not correspond to Clark's grouping which was based on cell type.

Much additional work must be done before we can understand the vagus and cervical sympathetics and the rôle which these mechanisms play in the regulation of heart rate, peripheral circulation and other vegetative functions.

SUMMARY

When the vagus nerve of the dog was cut proximal to the nodose ganglion and the cut fibers allowed to degenerate, two distinct groups of intact nerve fibers could still be demonstrated in the vago-sympathetic trunk.

The first of these fiber groups resembles the accelerator nerve of other investigators. Stimulation of this nerve causes an acceleration of the heart and a rise in blood pressure. It was demonstrated in 40 per cent of the animals studied.

The second group of fibers present in the vago-sympathetic trunk after vagotomy can probably be interpreted as being postganglionic parasympathetic fibers with their cells of origin in the nodose ganglia. Stimulation of these fibers results in a lowering of the blood pressure and sometimes a slowing of the heart. These fibers were demonstrated on the left side in 71 per cent of the cases studied and were present on the right side of 18 per cent of the cases used for a study of the right vagus.

BIBLIOGRAPHY

- ARLOING, S. 1896. *Arch. d. Physiol. Norm. et Pathol.*, viii, 75.
 BAYLISS, W. M. 1920. *Principles of general physiology*. London.
 BISHOP, G. H. AND P. HEINBECKER. 1930. *This Journal*, xciv, 171.
 BOEHM, R. 1875. *Arch. f. exp. Path. u. Pharm.*, iv, 255.
 CLARK, S. L. 1926. *Journ. Comp. Neurol.*, xli, 423.
 DALE, H. H., P. P. LAIDLAW AND C. T. SYMONS. 1910. *Journ. Physiol.*, xli, 1.
 GASKELL, W. H. 1920. *The involuntary nervous system*, p. 34.
 HERING, H. E. 1914. *Pflüger's Arch.*, cciii, 512.
 1924. *Pflüger's Arch.*, cciii, 100.
 KEN KURE, Y. N., T. MORIMASA, S. KENSAKU AND S. BINZI. 1928. *Quart. Journ. Exp. Physiol.* xviii, 333.
 KORIEŠA, L. 1931. *Zentralb. f. d. ges. Neurol. u. Psychiat.*, lix, 208.

MOLHANT, M. 1912. *Le Neuraxe*, xiv-xv, 525.

REED, C. I. AND J. A. LAYMAN. 1930. *This Journal*, xcii, 275.

SCHIFF, M. 1878. *Pflüger's Arch.*, xviii, 172.

SCHMIEDEBERG. 1871. Quoted in GARRISON's *History of medicine*, Philadelphia, 1921.

STEWART, G. N. 1918. *Manual of physiology*, p. 162.

TULGAN, J. 1923. *This Journal*, lxxv, 174.

WINKLER, C. 1918. *Manuel de Neurologie, Anatomie du Système Nerveux*. Haarlem.

STUDIES ON SUPRARENAL INSUFFICIENCY

X. DEPRESSOR RESPONSES TO SMALL DOSES OF ADRENALIN IN THE RAT, INDUCED BY LOSS OF THE SUPRARENAL MEDULLA

LELAND C. WYMAN AND CAROLINE TUM SUDEN

*From the Physiological Laboratory of Boston University School of Medicine and the
Evans Memorial, Mass. Memorial Hospitals, no. 809*

Received for publication April 11, 1932

The suggestion has been made in the past that adrenin may have a continuous effect maintaining the nutrition or tone of some part of the vascular neuro-muscular mechanism. For such a view we have found no evidence in our previous work; nor is it in accord with Cannon's recent report of "evidence that medulliadrenal secretion is not continuous" (1931). This suggestion, however, led us to examine the vascular responses to adrenalin of normal rats and of suprarenalectomized rats which were kept in good health by transplants of cortical tissue but had been lacking the suprarenal medulla for some time. In a survey of the effects of injected adrenalin on the vascular mechanism of a great variety of animals, Hartman, Kilborn and Lang (1918) came to the conclusion that rodents are exceptional in their reaction to adrenalin, having no vasodilator mechanisms sensitive to this substance. They could not obtain a fall of blood pressure in the white rat with adrenalin, responses to all effective doses being pressor. We confirmed this finding in urethanized, normal rats, but in the transplanted rats without suprarenal medulla the responses to small doses were consistently depressor. This phenomenon was so unusual that further work was done and the results are reported below.

METHODS. The general technique of studying suprarenal insufficiency in rats has been described in previous papers in this series. Details regarding methods of recording blood pressure, intravenous injections, and microscopic observation of blood vessels in rats may be found in the paper by Wyman and tum Suden (1932). Carotid blood pressures were measured by the mercury manometer method and intravenous injections were given in the femoral vein. Adult male rats, between 230 and 300 grams' body weight, were used throughout.

Normal rats. In a series of 17 normal male rats, anesthetized with urethane, with initial blood pressures ranging from 90 to 134 mm. Hg (average 105.4 mm. Hg), the minimal effective doses of adrenalin produced purely

pressor effects (fig. 1). In five cases such a dose was 0.05 cc. of 1:2,000,000, in nine cases 0.05 cc. of 1:1,000,000, in two cases 0.1 cc. of 1:1,000,000, and in one case 0.05 cc. of 1:4,000,000 produced a detectable pressor effect. In 10 rats anesthetized with ether with initial blood pressures from 102 to 118 mm. Hg (average 111.8 mm. Hg), similar minimal doses produced only pressor effects in four cases (fig. 12, A). In the other six cases the smallest effective doses (0.05 cc. of 1:2,000,000 to 0.05 cc. of 1:1,000,000) produced depressor responses, larger doses (0.05 cc. to 0.1 cc. of 1:1,000,000) produced pressor responses, and intermediate doses produced pressor-depressor effects (fig. 11, A). When depressor responses were obtained the fall of blood pressure produced by a given dose was reduced by decreasing the depth of the ether anesthesia just before the injection, and it was increased by administering more ether before the injection. Wyman and Lutz (1925) found that the pressor response to adrenalin in cats may be modified in various ways by varying the dosage of inhaled ether.

In these and in all subsequent experiments adequate control injections were given, of normal saline solution or of saline solution containing chloretone in amounts as great as or greater than that contained in the adrenalin chloride solution used (Parke, Davis & Co.).

Cortical transplants. In five suprarenalectomized rats having autoplasmic transplants of cortical tissue but no demonstrable chromaffin tissue and two suprarenalectomized rats having gross accessory cortical tissue, all exhibiting normal blood pressures under urethane anesthesia (104 to 122 mm. Hg, average 111.7 mm. Hg), definite depressor responses were regularly obtained with small doses of adrenalin, i.e., from 0.02 cc. to 0.1 cc. of 1:2,000,000 (figs. 2 and 3). Larger doses, from 0.1 cc. of 1:1,000,000 to 0.05 cc. of 1:500,000 or more, produced brief rises of blood pressure immediately followed by prolonged falls. Much larger doses, from 0.1 cc. of 1:500,000 to 0.05 cc. or 0.1 cc. of 1:100,000, were required to produce purely pressor effects. Exactly the same course of events was observed in three etherized suprarenalectomized rats having gross accessory cortical tissue, four months after operation (fig. 4). The depressor responses seen in these animals were much greater than any observed in normal rats under ether anesthesia, and large doses of adrenalin (0.1 cc. of 1:100,000), such as invariably produce marked pressor effects in etherized normal rats, caused pressor-depressor responses. Under urethane anesthesia, at least, there is apparently a complete reversal of the vascular responses to small doses of adrenalin in suprarenalectomized rats having cortical but no medullary suprarenal tissue. Such animals are perfectly healthy, have abundant body fat, and apparently differ from normal rats only in respect to the medullary defect.

Suprarenalectomized rats. Experiments were done with urethane anesthesia on nine suprarenalectomized rats which showed absence of all gross

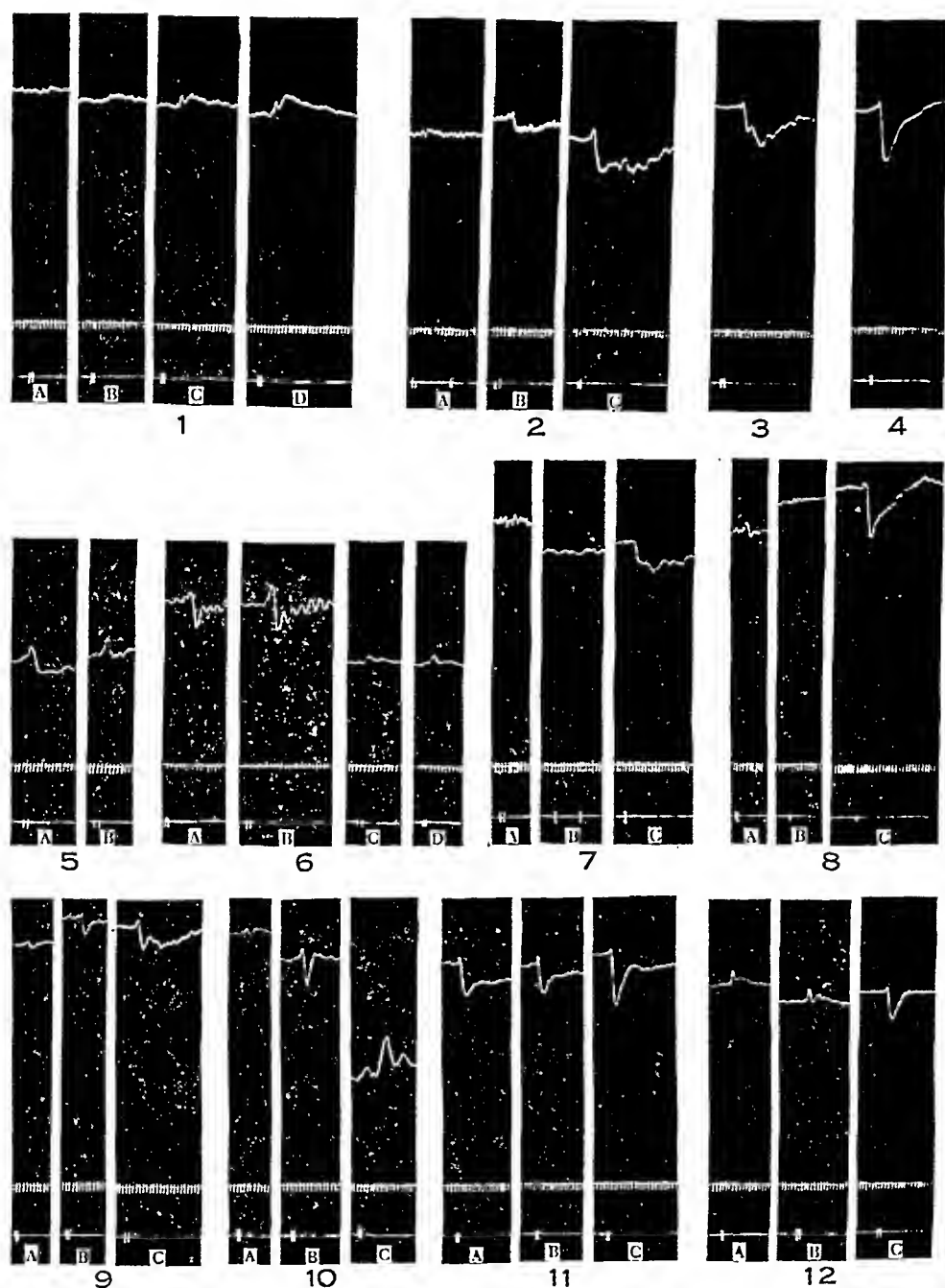


Fig. 1. Experiment 76. Normal male rat, 291 grams. Urethane anesthesia. In this, and in subsequent tracings, the time record shows five second intervals and is at zero blood pressure level. The lower record signals intravenous injections (femoral vein) of adrenalin solution or control injections of normal saline solution,

with or without chloretone. A, 0.1 cc. chloretone in normal saline, 1:500,000; B, 0.1 cc. adrenalin, 1:2,000,000; C, 0.1 cc. adrenalin, 1:1,000,000; D, 0.1 cc. adrenalin, 1:500,000.

Fig. 2. Experiment 62. Male rat, 276 grams, having transplanted cortical tissue but no demonstrable chromaffin tissue. Urethane anesthesia. A, 0.1 cc. and 0.05 cc. saline solution; B, 0.1 cc. adrenalin, 1:2,000,000; C, 0.05 cc. adrenalin, 1:500,000.

Fig. 3. Experiment 65. Male rat, 274 grams, transplant. Urethane anesthesia; 0.1 cc. adrenalin, 1:1,000,000.

Fig. 4. Experiment 98. Male rat, 298 grams, 124 days after suprarenalectomy, having gross accessory cortical tissue. Ether anesthesia; 0.05 cc. adrenalin, 1:1,000,000.

Fig. 5. Experiment 43. Male rat, 249 grams, 16 days after suprarenalectomy. Urethane anesthesia. A, 0.1 cc. adrenalin, 1:2,000,000; B, 0.1 cc. adrenalin, 1:1,000,000. Low blood pressure (52 mm. Hg), pressor action predominating.

Fig. 6. Experiment 38. Male rat, 301 grams, 10 days after suprarenalectomy. Urethane anesthesia. A, 0.05 cc. adrenalin, 1:1,000,000; B, 0.1 cc. adrenalin, 1:1,000,000 (blood pressure medium, 80 mm Hg; pressor-depressor action); C, 0.05 cc. adrenalin, 1:1,000,000; D, 0.1 cc. adrenalin, 1:1,000,000 (31 minutes after dose B; blood pressure low, 51 mm. Hg; pressor action only).

Fig. 7. Spinal cord transected under ether anesthesia, ether discontinued, blood pressure from femoral artery. A, Experiment 114. Normal male rat; 0.1 cc. adrenalin, 1:2,000,000, 44 minutes since discontinuing ether. B, Experiment 116. Male rat, 308 grams, transplant. 0.05 cc. and 0.1 cc. chloretone solution, 1:500,000, 38 minutes since ether. C, Experiment 116; 0.1 cc. adrenalin, 1:2,000,000, 46 minutes since ether.

Fig. 8. Decerebrated under ether anesthesia, ether discontinued, blood pressure from carotid artery. A, Experiment 119. Normal male rat, 304 grams; 0.1 cc. adrenalin, 1:1,000,000, 40 minutes since discontinuing ether. B, Experiment 118. Male rat, 260 grams, transplant. 0.1 cc. chloretone solution, 1:500,000, 35 minutes since ether. C, Experiment 118; 0.1 cc. adrenalin, 1:1,000,000, 54 minutes since ether.

Fig. 9. Experiment 87. Male rat, 285 grams. Urethane anesthesia. Suprarenals ligated and removed before the experiment. A, 0.1 cc. chloretone solution, 1:100,000, 90 minutes since suprarenalectomy. B, 0.1 cc. adrenalin, 1:2,000,000, 44 minutes since suprarenalectomy. C, 0.1 cc. adrenalin, 1:1,000,000, 48 minutes since suprarenalectomy.

Fig. 10. Experiment 97. Normal male rat, 240 grams. Urethane anesthesia. A, 0.1 cc. adrenalin, 1:1,000,000. Suprarenalectomy 20 minutes after dose A. B, 0.1 cc. adrenalin, 1:1,000,000, 16 minutes after suprarenalectomy. C, 0.1 cc. adrenalin, 1:1,000,000, 126 minutes after suprarenalectomy (blood pressure low, 54 mm. Hg; pressor action only).

Fig. 11. Experiment 108. Normal male rat, 278 grams. Ether anesthesia. A, 0.1 cc. adrenalin, 1:1,000,000. Control blank operation, 8 minutes later. B, 0.1 cc. adrenalin, 1:1,000,000, 9 minutes after blank operation. Suprarenals ligated 5 minutes after dose B. C, 0.1 cc. adrenalin, 1:1,000,000, 10 minutes after ligation of suprarenals. Increased depressor response.

Fig. 12. Experiment 110. Normal male rat, 250 grams. Ether anesthesia. A, 0.1 cc. adrenalin, 1:1,000,000. Control blank operation 5 minutes later. B, 0.1 cc. adrenalin, 1:1,000,000, 21 minutes after blank operation. Suprarenals ligated 45 minutes after dose B. C, 0.1 cc. adrenalin, 1:1,000,000, 9 minutes after ligation of suprarenals. Reversal of response.

suprarenal tissue at autopsy, from 10 to 17 days after operation in six cases and 99 or 101 days after operation in three cases. Such animals have a low blood pressure concurrent with the cortical defect (Wyman and tum Suden, 1932). In four cases with blood pressures between 38 and 58 mm. Hg only pressor responses to adrenalin were observed (fig. 5). The threshold dosage was the same as that for normal rats. In five cases with blood pressures between 60 and 80 mm. Hg depressor or pressor-depressor responses were obtained with the smallest effective doses (0.1 cc. of 1:2,000,000 or 1:1,000,000), and purely pressor effects with larger doses, as in the case of the transplanted rats, except that somewhat smaller doses sometimes produced purely pressor effects (fig. 6, A, B). This is consistent with the hypothesis proposed by Cannon and Lyman (1913), namely that the action of adrenalin on vascular smooth muscle varies according to the state of the muscle, being relaxation when the muscle is tonically shortened and contraction when relaxed. They observed that the depressor effect of small doses of adrenalin in the cat was reversed to pressor action when the blood pressure was lowered by various means. The gradation of depressor, through pressor-depressor, to purely pressor effects produced by the same dose of adrenalin in suprarenalectomized animals with different decreasing blood pressure levels is striking. That these differences are dependent upon the blood pressure level is further substantiated by reversal of the depressor to a pressor-depressor or pressor action in the same animal when the blood pressure falls during an experiment. Such a reversal was seen in eleven cases (fig. 6, C, D; fig. 10, C).

Acute experiments. In order to determine whether the reversal of the vascular response to a small dose of adrenalin is dependent upon a chronic condition developed in suprarenalectomized rats some time following operation or whether it appears immediately following the removal of the influence of the suprarenal medulla, a variety of experiments was done, using both urethane and ether anesthesia.

A control blank operation in the suprarenal sites accompanied by more trauma than that involved in double suprarenalectomy, or single suprarenalectomy together with a blank operation on the opposite side did not alter the blood pressure or the vascular responses to intravenous injections of various doses of adrenalin given from 2 to 72 minutes later (fig. 11, A, B; fig. 12, A, B). Such operations were done in ten cases, either before the blood pressure experiment was begun or during the recording of blood pressure after a number of test doses of adrenalin had been given.

Six urethanized rats were suprarenalectomized from 20 to 194 minutes before recording blood pressure. These animals had normal blood pressures and responded to intravenous adrenalin, injected from 35 to 209 minutes after suprarenalectomy, in the same way as the transplanted rats described above (fig. 9).

In a series of twelve rats, three urethanized and nine etherized, the suprarenals were carefully ligated during the experiment, after a number of test doses of adrenalin had been given. In six of these cases ligation of the suprarenals had been preceded by a control blank operation (from 12 to 79 minutes previously), so that the necessary incisions had already been made and the ligation involved a minimum of operative trauma. In these cases also, the normal control injections of adrenalin, those following blank operation, and those following suprarenal ligation were all carried out in succession in the same animal and were thus quite comparable. In all cases simple ligation of the suprarenal glands, with no accompanying alteration of blood pressure, was followed by reversal of the vascular responses to small doses of adrenalin. Doses which had produced purely pressor responses before the ligation and whose action had not been altered by the control operation now produced definite depressor responses (figs. 10 and 12). The reactions to increasing doses of adrenalin, were the same as those seen in the transplanted rats or in rats suprarenalectomized shortly before the blood pressure experiment. In four cases, under ether anesthesia, the smallest doses had produced depressor responses before suprarenal ligation. In these cases, following the ligation, similar doses produced much greater depressor responses, the fall of blood pressure sometimes being twice as great as previously (fig. 11). Depressor responses to small doses were obtained from 2 to 108 minutes after ligation of the suprarenals. Evidently the reversal occurs immediately following the removal of the influence of the suprarenal medulla, and is constant for a comparatively long time. Judging from the data obtained from transplanted animals, tested four months after suprarenalectomy, it is a permanent reversal.

Experiments without anesthesia. In 1926 Macdonald and Schlapp reported that in the unanesthetized decerebrate cat no depressor responses to small doses of adrenalin could be evoked, and in 1928 Dragstedt, Wightman and Huffman found that in the normal unanesthetized dog the minimal effective dose of adrenalin on sustained administration produces pressor effects. The opinion became prevalent, therefore, that the depressor response to a small dose of adrenalin is an effect conditioned by anesthesia. Gruber (1928) found, nevertheless, that small doses of adrenalin cause a fall of blood pressure in decerebrated cats from 30 minutes to 3½ hours after withdrawal of the anesthetic. It became necessary, therefore, to see if the depressor response in rats lacking the suprarenal medulla is obtained only in anesthetized animals.

Two types of experiment were done. In one the spinal cord was sectioned in the mid-lumbar region under ether anesthesia, the rat was fastened as comfortably as possible to an operating board, the ether was discontinued, and blood pressure was recorded from the femoral artery. In the other the

mid-brain was sectioned under ether anesthesia through a small hole in one side of the skull after ligating the carotid arteries, the ether was discontinued, and blood pressure was recorded from the carotid artery as usual. Both types were done in the case of normal and of suprarenalectomized rats having autoplasmic cortical transplants but no medullary tissue three months after transplantation. In all cases the blood pressures were within the normal range after preparation of the animal and during the experiment (104 to 136 mm. Hg).

The reactions to various amounts of adrenalin injected intravenously were the same in all cases as those observed in urethanized rats and described above. In normal rats the minimal effective doses, given from 25 to 144 minutes after discontinuing ether, were purely pressor in action and were of the same general magnitude as those for anesthetized rats. In transplanted rats small doses, given from 32 to 64 minutes after discontinuing ether, caused depressor responses, and the size of the effective doses and of the depressor responses were again similar to those characteristic of anesthetized rats (figs. 7 and 8). Evidently the phenomenon in question is not conditioned by anesthesia.

Heart rate. Records were taken on a fast drum during various types of experiment and in the case of the different types of animal. Counts showed that no significant changes in heart rate were caused by the control injections of saline or of chloretone solutions, or by the small doses of adrenalin.

Direct observation of blood vessels. Direct microscopic observation of the blood vessels of the ear by transmitted light and of the vessels of the intestines by reflected light through a celluloid abdominal window (Mendenhall, 1931) was carried out on normal and on transplanted rats during the intravenous injection of various amounts of adrenalin. In the case of the ear vessels of both types of rat only vasoconstriction (veins and arteries) was observed, and the minimal effective dose of adrenalin was 0.1 cc. of 1:500,000. In the case of the vessels on the surface of the intestine or in the mesentery of normal rats the minimal dose which caused changes of sufficient magnitude to be definitely interpreted was 0.05 cc. of 1:500,000, and produced constriction only. Larger doses, from 0.1 cc. of 1:500,000 to 0.05 cc. of 1:10,000, produced constriction only or predominating constriction followed by slight dilatation. In transplanted rats doses of from 0.1 cc. of 1:1,000,000 to 0.1 cc. of 1:100,000 caused brief constriction followed by a marked, prolonged dilatation. Larger doses caused marked constriction followed by a comparatively brief dilatation. Evidently the depressor phenomenon induced by loss of the suprarenal medulla is of vascular origin. The observations on the normal rats are in accord with those of Hoskins, Gunning and Berry (1916) on the dog with respect to cutaneous vessels, and are similar to those of Hartman and McPhedran

(1917) on dogs and cats, namely, that small doses cause constriction of intestinal vessels while larger doses cause constriction followed by dilatation.

DISCUSSION. From the results reported above it may be concluded that the generalization stating that rodents do not possess vasodilator mechanisms sensitive to adrenalin is not strictly accurate. Although normal rats under urethane anesthesia or without anesthesia do not react to minimal effective doses by vasodepression, they occasionally show depressor responses to adrenalin under ether anesthesia. The depressor responses to small doses of adrenalin induced by loss of the suprarenal medulla indicate, moreover, that vasodilator mechanisms responding to adrenalin are present, in the rat at least, although their presence in the intact animal is usually masked by pressor reactions. The microscopic observations of blood vessels indicate that these depressor responses are caused by peripheral vascular dilatation, the splanchnic vessels but not the skin vessels being involved. No observations were made on the vessels of skeletal muscle. Experiments without anesthesia show that the responses are not dependent upon the presence of an anesthetic. From this work and from the work of others on decerebrated animals it may be concluded that it is not safe to make the generalization that all depressor responses to adrenalin are conditioned by anesthesia.

An attempt at explanation of the reversal of the vascular response in the rat to a small dose of adrenalin occasioned by removing the suprarenal medulla is difficult, because there is no general agreement as to the cause of depressor responses to adrenalin in other animals. As stated above, the results seen in suprarenalectomized and in other types of rats with low blood pressures are consistent with the hypothesis of Cannon and Lyman (1913) that the action of small amounts of adrenalin varies according to the state of the vascular smooth muscle. Hartman and his collaborators concluded that adrenalin has a differential action on various parts of the vascular tree, and that depressor responses after small doses may be explained by the predominance of vasodilatation in certain areas over vasoconstriction in others (see Hartman, 1918). There is much evidence from various sources to support the idea of the existence of sympathetic vasodilator depressor mechanisms.

The reversal from pressor to depressor action appears a few minutes after removal of the influence of the suprarenal medulla, and is apparently permanent. It might be postulated that in the intact animal the injection of a minute dose of adrenalin causes the immediate secretion of enough additional adrenin from the rat's own glands to make the resulting response pressor in nature. The brief latent period of the response, however, militates against an explanation involving the induction of secretory processes. Much work remains to be done before this phenomenon can be explained, and it is quite possible that some undetected factor other than removal of

the suprarenal medulla is present. As a working hypothesis, one involving differential change in irritability of pressor and depressor mechanisms seems more attractive.

There is no doubt that a great many factors, many of them chemical in nature, can modify the vascular reactions to adrenalin (Collip, 1922; Lutz and Wyman, 1925; Wyman and Lutz, 1925). Many of these probably operate by changing the irritability of some part of the peripheral neuro-muscular mechanism in a selective manner. Hoskins and Rowley (1915) found that intravenous infusion of adrenalin decreases the irritability of both the pressor and depressor mechanisms of the dog. They also found that the depression of irritability develops almost immediately after beginning the infusion, and that it disappears as quickly upon discontinuing the infusion. Although Cannon (1931) has recently presented evidence that in the normal resting animal adrenin is not secreted continuously, he points out that operative disturbance causes a considerably exaggerated medulliadrenal secretion; and that "on abolishing, by adrenalectomy or cutting the secretory nerves, the extra secretion induced under experimental conditions, characteristic atonic effects might result because the spontaneously secreted adrenin has been exerting a tonic influence." It is conceivable, therefore, that in the experiments reported above such a secretion of adrenin in normal rats, induced by the experimental procedure, is constantly depressing the irritability of the vasomotor mechanisms. If we assume that the vaso-depressor mechanisms may be more profoundly affected than the pressor mechanisms, then removal of the suprarenals should allow their action to be more easily elicited, and depressor responses to small doses of adrenalin would be more likely to occur. Hence the reversal following ligation of the glands. In transplanted rats, with no medullary tissue, no such secretion of adrenin can occur, hence depressor irritability is not differentially decreased and depressor responses may be regularly obtained. As indicated above, such a conception is offered merely as a tentative hypothesis.

SUMMARY

1. In urethanized and in unanesthetized normal rats minimal effective intravenous doses of adrenalin produced only pressor responses. In etherized normal rats similar minimal doses occasionally produced depressor effects.

2. In suprarenalectomized rats having transplants of cortical tissue but no demonstrable chromaffin tissue (either urethanized, etherized or unanesthetized), small doses of adrenalin produced depressor responses, larger doses produced pressor-depressor responses and much larger doses were required to produce purely pressor effects.

3. In suprarenalectomized rats similar results were obtained unless the blood pressure was low, when pressor responses alone were seen.

4. Removal or simple ligation of both suprarenal glands was immediately followed by reversal from pressor to depressor of the vascular responses to small doses of adrenalin. The reactions to increasing doses were the same as those in transplanted rats.

5. No significant changes in heart rate were observed. Microscopic observation of blood vessels during the injection of adrenalin in normal and in transplanted rats showed only constriction of the ear vessels, predominating constriction of the intestinal vessels in normals, and brief constriction followed by marked dilatation of intestinal vessels in transplants.

BIBLIOGRAPHY

- CANNON, W. B. 1931. *This Journal*, xcvi, 447.
CANNON, W. B. AND H. LYMAN. 1913. *This Journal*, xxxi, 376.
COLLIP, J. B. 1922. *Endocrinol.*, vi, 402.
DRAGSTEDT, C. A., A. H. WIGHTMAN AND J. W. HUFFMAN. 1928. *This Journal*, lxxxiv, 307.
GRUBER, C. M. 1928. *This Journal*, lxxxiv, 345.
HARTMAN, F. A. 1918. *Endocrinol.* ii, 1.
HARTMAN, F. A., L. G. KILBORN AND R. S. LANG. 1918. *Endocrinol.*, ii, 122.
HARTMAN, F. A. AND L. MCPHEDRAN. 1917. *This Journal*, xliii, 311.
HOSKINS, R. G., R. E. L. GUNNING AND E. L. BERRY. 1916. *This Journal*, xli, 513.
HOSKINS, R. G. AND W. N. ROWLEY. 1915. *This Journal*, xxxvii, 471.
LUTZ, B. R., AND L. C. WYMAN. 1925. *This Journal*, lxxii, 488.
MACDONALD, A. D. AND W. SCHLAPP. 1926. *Journ. Physiol.*, lxii, xii.
MENDENHALL, W. L. 1931. *Science*, lxxiv, 245.
WYMAN, L. C. AND B. R. LUTZ. 1925. *This Journal*, lxxiii, 254.
WYMAN, L. C. AND C. TUM SUDEN. 1932. *This Journal*, xcix, 285.

THE ELASTICITY OF THE DURAL SAC AND ITS CONTENTS

LOUIS B. FLEXNER, JANET H. CLARK AND LEWIS H. WEED

From the Departments of Anatomy and Physiological Hygiene, Johns Hopkins University

Received for publication April 13, 1932

A report, recently published by the writers (Weed, Flexner and Clark, 1932), presented evidence of the existence of an important relationship between the dislocation of cerebrospinal fluid and its pressure. This relationship was developed from information yielded by experiments dealing with the abrupt tilting of dogs from the horizontal to the vertical head-down and tail-down positions. In these former observations, the pressure of the cerebrospinal fluid in the occipital region was determined by successive use of the so-called "bubble manometer" and of open-end manometers of various bores. With the bubble manometer pressure-changes in the cerebrospinal fluid were recorded without external dislocation of fluid, but with the open-end series an obvious dislocation of fluid into or from the manometer occurred on such tiltings. Determination of the volume of fluid displaced into or from the external system showed that as a progressively larger dislocation of fluid occurred in the manometers of larger and larger bore, the pressure-changes in these manometers became smaller and smaller.

From the data assembled in these tilting experiments, it was found that the difference in volume of displaced fluid had a definite relationship to the difference in pressure-change. This relationship was expressed in the fraction $\frac{dV}{dP}$, where dV represented the difference in volume-change between the tilting with no external displacement (bubble manometer) and the actual cubic centimeter change in any of the open-end manometers, while dP represented the difference in centimeters of the pressure-change on tilting between that recorded by the bubble manometer and those of any of the open-end manometers. Calculation of this fraction $\frac{dV}{dP}$ gave a fairly constant value in experiments carried out on dogs of a uniform size (weight, 6-7 kgm.). The fraction, which in dogs of that size was found to have an average value of 0.17, was also derived by using the differences in the pressure-changes from any two of the open-end manometers for comparison with the corresponding volume-differences. Employment of

this factor $\frac{dV}{dP}$ with its average value enabled the writers accurately to determine in young dogs of the same size the decrease in the intradural contents effected by the intravenous injection of strongly hypertonic saline solutions (Weed and McKibben, 1919a, b).

It was pointed out in the previous report that this fraction $\frac{dV}{dP}$ was related to the general physical formula for an elastic system. The expression for the coefficient of elasticity of a solid, fluid or gas, is the quotient obtained by dividing the stress by the strain. In this formula for the coefficient of the volume-elasticity E , the stress is the change in pressure dP , and the strain is the change in volume dV divided by the original volume V ; i.e., $E = \text{coefficient of elasticity} = dP / \left(\frac{dV}{V} \right) = \frac{dP}{dV} V$.

It was found that this formula could be applied to the dural sac and its contents as the volume-elasticity of the membranes and vascular bed apparently led to a dislocation of fluid dV which is related to a pressure-change dP on tilting. As the ratio $\frac{dV}{dP}$ was ascertained to be fairly constant in any one animal, where the total volume V was the same, the formula given above may be applied to calculation of the coefficient of elasticity when $\frac{dV}{dP}$ and V have been determined. This led us to compute the elasticity of the dural sac and its contents in the dog. The first step in each experiment consisted in the derivation of the value of the fraction $\frac{dV}{dP}$ for the individual animal by measurement of the pressure-changes in the cerebrospinal fluid, with manometers of different bore, on tilting from the horizontal to the vertical head-down and tail-down positions. This procedure was followed by measurement of the volume of the animal's intradural contents, both cranial and spinal. These determinations $\left(\frac{dV}{dP} \text{ and total volume } V \right)$ enabled us to derive the coefficient of elasticity in dynes per square centimeter by substitution in the formula $E = V / \frac{dV}{dP}$.

METHODS OF INVESTIGATION. While in the previous series of experiments dogs of a uniform size (6-7 kgm.) were employed, the present series of observations is based upon dogs of various sizes and various ages. The dogs were graded, as far as possible, as immature, young adult, adult and old

¹ Pressure was measured in centimeters of normal saline solution (or cerebrospinal fluid); but in order to give the coefficient of elasticity in C. G. S. units, pressure which is defined as force (mass \times acceleration) per unit area, must be expressed as dgh (density \times acceleration of gravity \times height in centimeters).

animals. They were all anesthetized with ether, given by Woulffe bottle with intratracheal tube, and were then firmly fixed to a tilting table. The pressure of the cerebrospinal fluid was measured by attachment of open-end manometers of various bores (1 mm., 4 mm., 6 mm., 8 mm., and 10 mm.) to a needle inserted through the occipito-atlantoid ligament into the subarachnoid space. Initially the 1 mm. manometer was connected to the puncture-needle and the animal tilted from the horizontal to the two vertical positions. The readings of this manometer were followed by the attachment of the other manometers, with similar tiltings carried out with each manometer. The pressure-changes in the two series of vertical tilts (head-down and tail-down) were thus determined and the volume of fluid dislocated into or from each manometer calculated from the known capacities of the manometers.

The bubble manometer, as used in the former experiments, was not employed in these observations, because of additional technical difficulties during the tiltings. The pressure-changes in the cerebrospinal fluid, obtained with the open-end 1 mm. manometer, were considered as the baseline for reference to the pressure-changes recorded by the manometers of larger bore.

At the conclusion of each experiment, the animal was killed and the total intradural volume determined. The simplest method of accurate measurement of this volume was found to consist first in the removal of the head from the vertebral column. The intradural contents of the cranium were washed out with a fine stream of water, after first destroying the integrity of the brain by a stiff wire. For the spinal determinations, the best procedure was found to consist initially of laminectomy in the lower lumbar and mid-thoracic regions, taking care not to injure the spinal dura. In the first few animals a complete laminectomy was done but it was later found that exposure in these two segments was sufficient. The speedy maceration of the spinal cord and the spinal leptomeninges was then achieved by pushing a blunt fusiform enlargement of a fairly stiff wire through the entire length of the spinal dural canal, after first opening the dural sac in the lower lumbar segment. The macerated tissues were next completely washed out by a jet of water from a fine rubber tube.

After complete removal of the substance of the nervous system, the cranial intradural cavity was filled with mercury and the contained mercury subsequently measured. This method was found to be accurate, though signs of undue pressure, due to the weight of the mercury, were occasionally apparent. The method was checked for accuracy in a few animals by filling the cavity with lead shot with later measurement of the volume of these shot.

The early determinations of the spinal intradural volume were made by this method of filling the dural sac with shot. The technical difficulties

of this method were so great that mercury was next tried. The mercury was found to be too heavy for this purpose, and finally the simple expedient of inserting a small cannula into the lower end of the lumbar dural sac was employed. Through this cannula into the spinal dural tube a colored mineral oil was allowed to run from a burette, the animal being held with the head-end elevated until the filling was complete. This method checked accurately against the laborious employment of lead shot in such wet preparations.

EXPERIMENTAL FINDINGS. It was early discovered that a constant degree of light surgical anesthesia was necessary for the successful completion of the experiments with repeated tilting. In the customary experiment the necessary adjustment of the ether was early achieved and accurate measurements of the pressure-changes of the cerebrospinal fluid were then obtained. Because of frequent disturbances in respiration (chiefly hyperpnea) when the animals were in the vertical positions, it was found expedient to pinch off the ether tube during these periods. With care taken to secure a uniform degree of light surgical anesthesia and elimination of difficulties due to increasing etherization in the vertical positions, experiments yielding comparable results were readily carried to completion.

With the open-end manometer of 1 mm. bore attached at the beginning of the experiments, tiltings from the horizontal to the two vertical positions were made at least twice for each position. When experimental conditions were well established, the pressure-changes in the cerebrospinal fluid on such tiltings were found to be quite similar, usually varying but 2 or 3 mm. In cases of marked variation in the pressure-changes, several additional tiltings were performed so that an average value for the pressure-change could be obtained. In each animal, after attachment of the next manometer in series, at least two tiltings to each of the two vertical positions were made before proceeding to the next larger manometer.

With the pressure-changes and the volume-changes determined for each of the manometers, it became possible to calculate the fraction $\frac{dV}{dP}$ for the individual animal. This derivation was made by taking as dP the difference between the pressure-change obtained by the use of the 1 mm. manometer on tilting, and the pressure-changes recorded by the other manometers. The volume-change dV represented the difference between the volume displaced in the 1 mm. manometer on tilting and that of any of the larger open-end manometers. An example of this derivation of the fraction $\frac{dV}{dP}$ is given in table 1.

With the dogs classed as accurately as possible as immature, young adult, adult and old animals, it was found that the value of the fraction $\frac{dV}{dP}$

varied from 0.132 in an immature dog of 3180 grams body-weight, to 0.272 in an old dog of 14,545 grams. The intradural volume in the same series of animals ranged between 60.8 cc. and 105.1 cc. There was apparently no relationship between spinal length (occipital protuberance to last lumbar spine), body-weight, and intradural volume.

When calculations of the coefficient of elasticity² were made by substituting in the formula the values of $\frac{dV}{dP}$ and of intradural volume V for each animal as shown in table 1, it was discovered that the various groups of animals showed an extraordinary constancy in the value of the coefficient

TABLE 1
Derivation of $\frac{dV}{dP}$ and E for dog C-37

MANOMETER	HEAD-DOWN					TAIL-DOWN				
	Pressure-change C.S.F.	Difference in pressure-change	Volume dis-located	Difference in volume dis-located	$\frac{dV}{dP}$	Pressure-change C.S.F.	Difference in pressure-change	Volume dis-located	Difference in volume dis-located	$\frac{dV}{dP}$
mm.	cm.	cm.	cc.	cc.		cm.	cm.	cc.	cc.	
1	15.4		0.267			8.1		0.141		
4	10.4	5.0	1.009	0.742	0.148	5.6	2.5	0.543	0.402	0.161
6	6.7	8.7	1.769	1.502	0.172	3.3	4.8	0.871	0.730	0.152
8	4.0	11.4	2.040	1.773	0.156	2.1	6.0	1.071	0.930	0.155
10	2.9	12.5	2.067	1.800	0.145	1.5	6.6	1.069	0.928	0.141
Average.....					0.155					0.152

$$\text{Average } \frac{dV}{dP} = 0.154.$$

$$\text{Intradural volume} = 63.1 \text{ cc.}$$

$$E = V \frac{dV}{dP} = \frac{63.1}{0.154} = 409 \times 980 \times 1.006 = 4.03 \times 10^5 \text{ dynes per cm.}^2$$

of elasticity. This constancy can be best portrayed in tabular form for the series of 20 animals. In this table 2, the coefficient of elasticity for the immature group is greater in dynes per cm.² than it is in the group of young adults. The adult animals in turn show a somewhat smaller coefficient of elasticity than do those animals graded as young adults, and the obviously old animals of the series show the lowest coefficient of elasticity. In the series portrayed, one dog is obviously out of line with the others of his age-group. This is animal C-36, which was classed as an adult but

² This E is a general elastic coefficient for the system as a whole and should not be confused with the coefficient of linear stretch (Young's Modulus) of the membranes and owing to the complexity of the system cannot be directly related to it.

whose coefficient of elasticity would place it in the upper end of the group of young adults. With this exception, the results given in the table for the coefficient of elasticity of the dural sac and its contents exhibit a surprisingly small variation from animal to animal.

Table 2 is compiled from the data obtained only in those experiments where the writers believe that no experimental errors had occurred. Of all the dogs subjected to this experimentation, only three animals were excluded because of such errors. The first of these animals was a small

TABLE 2
Coefficient of elasticity of the dural sac and its contents

EXPERIMENT NO.	WEIGHT	SPINAL LENGTH	INTRADURAL VOLUME	$\frac{dV}{dP}$	ELASTICITY	AGE-GROUP
	grams	mm.	cc.		dynes per cm. ²	
C 28	3,180	315	61.9	0.132	4.62×10^5	Immature
C 31	3,250	320	65.0	0.138	4.63×10^5	Immature
C 32	3,440	315	65.1	0.142	4.52×10^5	Immature
C 17	4,520	365	60.8	0.142	4.22×10^5	Young adult
C 20	5,900	390	63.7	0.147	4.26×10^5	Young adult
C 25	7,300	437	67.7	0.152	4.39×10^5	Young adult
C 16	5,074	373	67.0	0.157	4.20×10^5	Young adult
C 18	4,550	350	66.5	0.158	4.15×10^5	Young adult
C 27	4,435	395	70.3	0.163	4.25×10^5	Young adult
C 33	5,350	405	73.0	0.176	4.09×10^5	Young adult
C 35	7,520	435	76.7	0.184	4.16×10^5	Young adult
C 37	8,090	408	63.1	0.154	4.03×10^5	Adult
C 38	4,390	415	72.2	0.177	4.02×10^5	Adult
C 15	7,020	435	73.0	0.178	4.03×10^5	Adult
C 12	4,270	387	75.7	0.186	4.02×10^5	Adult
C 36	13,182	465	88.1	0.197	4.41×10^5	Adult
C 24	9,200	570	94.4	0.230	4.03×10^5	Adult
C 47	8,640	435	73.2	0.188	3.84×10^5	Old
C 45	16,004	482	82.6	0.216	3.78×10^5	Old
C 29	14,545	530	105.1	0.272	3.82×10^5	Old

dog (C-11) in which, because of technical difficulties with the occipital puncture, pressure readings could not be made with accuracy. The second animal (C-19) was excluded because of error in the determination of the intradural volume due to a long tear in the spinal dura mater. The last animal excluded from the table was dog C-21, the findings in which were considered unreliable because of inaccurate determination of the value of the fraction $\frac{dV}{dP}$. With the exception of these three animals, the data from all dogs in the series are included in table 2.

As pointed out in the previous report, an occasional dog showed a marked difference in the value of the fraction $\frac{dV}{dP}$ for the vertical head-down and tail-down tilts. Only one such dog had been found in the previous series, and because of the striking difference in the magnitude of the fraction for the two tiltings the record was included in the previous publication (Weed, Flexner and Clark, 1932) as table 2. In the series of 20 animals shown in table 2 of this report, this phenomenon of difference in magnitude of the fraction $\frac{dV}{dP}$ on head-down and tail-down tiltings was found in 4 animals out of 20. In these animals the head-down values of $\frac{dV}{dP}$ were employed for the determination of the coefficient of elasticity; the tail-down values of $\frac{dV}{dP}$ for these four animals were in general approximately one-half of the head-down values. This finding indicates a difference in elasticity for the two vertical positions in the occasional dog (one out of five). In general, however, the tiltings from the horizontal to the vertical head-down position gave values of the fraction $\frac{dV}{dP}$ quite similar to those obtained on tilting from the horizontal to the vertical tail-down position. Thus, in animal C-27, the head-down tiltings gave a value of 0.162 to the fraction while the contrariwise tiltings yielded a value of 0.165. Again, in animal C-36, the head-down value of $\frac{dV}{dP}$ was 0.197 and the tail-down value 0.196; while in dog C-37, the head down value of $\frac{dV}{dP}$ was 0.155 and the tail-down value 0.152. As a general rule the head-down values of $\frac{dV}{dP}$ were slightly in excess of the tail-down values, but occasionally animals yielded the opposite finding, as in animal C-45, where the head-down tiltings resulted in a value of the fraction $\frac{dV}{dP}$ as 0.201, while the tail-down value was 0.224.

In the determination of the values of $\frac{dV}{dP}$, the four calculations for the head-down tiltings were averaged; and similarly the four calculations for the tail-down tiltings. This procedure resulted in a far more accurate determination of the fraction than any one reading would necessarily give; but when experimental conditions were satisfactory, with no variation in the anesthesia, it was amazing to find how close the individual values of $\frac{dV}{dP}$ would fall (see table 1).

With this determination of the coefficient of elasticity for the various

age-groups of dogs, it becomes possible to calculate the intradural volume for any animal from its individual value for $\frac{dV}{dP}$. Such calculations have been made in table 3 which is composed of data obtained from the same dogs recorded in table 2. The calculated values in most cases differ but slightly from the actual determined values, even in the group of young

TABLE 3
Calculated and measured intradural volumes

The calculation for the intradural contents is made by substituting known values in the formula $E = V \frac{dV}{dP}$ or $V = E \frac{dV}{dP}$. In this substitution dP = height in centimeters \times acceleration of gravity (980) \times density (1.006).

EXPERIMENT NO.	AVERAGE ELASTICITY	$\frac{dV}{dP}$	CALCULATED INTRADURAL VOLUME	MEASURED INTRADURAL VOLUME	DIFFERENCE FROM CALCULATED VOLUME	AGE-GROUP
	<i>dynes per cm.²</i>		<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	
C 28	4.58×10^5	0.132	61.4	61.9	+0.5	Immature
C 31	4.58×10^5	0.138	64.2	65.0	+0.8	Immature
C 32	4.58×10^5	0.142	66.0	65.1	-0.9	Immature
C 17	4.22×10^5	0.142	60.8	60.8	0	Young adult
C 20	4.22×10^5	0.147	62.9	63.7	+0.8	Young adult
C 25	4.22×10^5	0.152	65.1	67.7	+2.6	Young adult
C 16	4.22×10^5	0.157	67.2	67.0	-0.2	Young adult
C 18	4.22×10^5	0.158	67.6	66.5	-1.1	Young adult
C 27	4.22×10^5	0.163	69.8	70.3	+0.5	Young adult
C 33	4.22×10^5	0.176	75.3	73.0	-2.3	Young adult
C 35	4.22×10^5	0.184	78.7	76.7	-2.0	Young adult
C 37	4.03×10^5	0.154	63.0	63.1	+0.1	Adult
C 38	4.03×10^5	0.177	72.4	72.2	-0.2	Adult
C 15	4.03×10^5	0.178	72.8	73.0	+0.2	Adult
C 12	4.03×10^5	0.186	76.1	75.7	-0.4	Adult
C 36	4.03×10^5	0.197	80.6	88.1	+7.5	Adult
C 24	4.03×10^5	0.230	94.1	94.4	+0.3	Adult
C 47	3.81×10^5	0.188	72.7	73.2	+0.5	Old
C 45	3.81×10^5	0.216	83.6	82.6	-1.0	Old
C 29	3.81×10^5	0.272	105.3	105.1	-0.2	Old

adult dogs where the maximum difference between the calculated and measured values is 2.6 cc. in experiment C-25; this maximum variation is approximately 4 per cent. In the group of adult dogs where the average is based on five animals whose coefficients of elasticity were either 4.02 or 4.03×10^5 dynes per cm.², the one animal out of line (C-36) showed a difference between calculated and determined values of the intradural contents of 7.5 cc., an error of less than 10 per cent.

Other types of calculation may similarly be made by substitution in the general formula for the coefficient of elasticity. If the total volume of the intradural contents is known, it is possible to calculate the value of the fraction $\frac{dV}{dP}$ on the basis of the average coefficient of elasticity for that age-group. Quite similarly, if the value of the fraction $\frac{dV}{dP}$ is determined for any animal or for those of similar size and age (where the volume V may be assumed to be the same), any change in elasticity may easily be ascertained. Likewise, if the value of $\frac{dV}{dP}$ is known for any one animal or group of the same size and age, any change in the pressure of the cerebrospinal fluid (dP) will permit a calculation of the volume-change (dV) of the intradural contents (as when the nervous system is decreased in volume by dehydration).

DISCUSSION. The data presented in the foregoing pages indicate that it is possible to obtain a coefficient of elasticity of the cerebrospinal system of living animals by application of the usual physical formula for such determinations. Within the various age-groups of dogs, the coefficient of elasticity was found under satisfactory experimental conditions to be of considerable constancy, the immature animals giving values of approximately 4.6×10^5 dynes per cm.², while the old animals yielded values in the neighborhood of 3.8×10^5 dynes per cm.² Between these two limits occurred the coefficients of elasticity of the groups of animals classed as young adults and adults. The reason for the apparent separation of the values of the two groups at the extremes of the classification lies in the fact that an attempt was made to secure for these two groups a few animals obviously immature though of suitable weight, and a few animals obviously old. Were the series of dogs to be even more widely extended, covering animals of all ages from birth to senility, the values of the coefficient of elasticity would undoubtedly merge gradually from one group into the other.

It seems very remarkable that a physical formula used for the determination of the coefficient of elasticity of solids, fluids, and gases on compression, could be applied with profit to the problem of meningeal elasticity so that the relationship of the external dislocation of cerebrospinal fluid to its resultant pressure might be expressed as a fraction $\frac{dV}{dP}$ with a constant value for any one animal. In the case of the central nervous system, it is obvious that we are not dealing with the phenomenon of compression of the contents of the intradural cavity nor with the possible dilatation of these contents under conditions of reduced pressure. Rather are we here dealing with the elasticity of the membranes and vascular bed

which permits an internal shift of fluid from one part of the nervous system to another and an external dislocation into or from manometers under conditions of positional change. In the experiments under discussion the total volume of the intradural contents may be taken to vary only to the extent of the amount of dislocation of fluid into or from the manometer as any intradural volume-change of the cerebrospinal fluid is at least in part compensated for by reciprocal variation in the total amount of blood within the dural sac. This reciprocal compensation in the blood vascular volume cannot be complete or the total volume V would not vary by the amount dV and the formula for elasticity could not be applied. But by means of the reciprocal compensation in the vascular bed, the internal readjustment in volume is kept constant enough, throughout the range of the experiments, to insure a constant relationship between external volume and pressure-changes. Such a statement of course implies a constancy in the volume of the true central nervous tissue itself (brain and spinal cord), but under the conditions of experimentation these two structures may be taken to be of unchanging volume.

In addition to such reciprocal compensation as may occur within the blood vessels of the meninges and central nervous system, there is a strong likelihood of somewhat analogous compensatory phenomena occurring within the venous plexus of the epidural space. Such changes in these thin-walled vessels would play but little rôle when the spinal dura is put under additional pressure as in the tail-down tilts, but in the contrariwise positional tiltings the dilatation of this extensive venous bed would permit an inward collapse of the spinal dural tube such as would not be possible without an elastic element within the epidural space.

While emphasis has been placed upon the dislocation of cerebrospinal fluid in the pressure-changes ensuing upon abrupt positional change in these dogs, it is realized that this fluid is merely a medium in affording a means of pressure-change and volume-change. The pressure-change dP , while existing in the fluid itself, is actually reflected by the fluid upon all of the intradural structures, so that all parts of this cerebrospinal system are affected in some way by the recorded pressure-change. Somewhat similarly the volume-change dV represents not an alteration in the total volume of the cerebrospinal fluid (though in the experiments the calculation of dV is made solely upon the fluid) but an alteration in the total volume of the contents of the dural envelope (cranial and spinal), whether of nervous tissue itself, of blood vascular bed, or of the cerebrospinal fluid. Because of these considerations the total volume of the intradural contents has been taken to represent the volume V in the formula used.

Attention has been given to the fact that throughout the period of experimentation there must be a continuous production and absorption of cerebrospinal fluid. This production of fluid, with its equivalent absorp-

tion, does not seem to modify the relationship between the dislocation of fluid and its pressure, even though the pressure-reactions suggest that the absorption of fluid is more rapid in the head-down position than in the horizontal and tail-down positions. Any difficulties in this regard are apparently eliminated by our practice of determining the pressure-changes in the cerebrospinal fluid with reference to the pressure in the horizontal position before tilting rather than to the pressure recorded on return to the position (cf. Weed, 1929a, b). Uniform procedures followed for all the manometers would yield comparable results and would eliminate any possible errors from these factors of production and absorption.

The question naturally arises as to the exact meaning of the term coefficient of elasticity, when applied to such a complex organization as that of the cranio-vertebral contents. Elasticity, to accept the physicists' definition, is power to resist change in shape, or power to resume the original shape after deformation. It follows therefore that, under a given change in pressure, the greater the change in volume, the smaller is the coefficient of elasticity. The high values in dynes, obtained for the coefficient of elasticity of the cerebrospinal system in immature animals, indicate that more pressure is required to effect a given change than is required for the old animals of the series. This means that in these immature animals the power of the cerebrospinal system to resist deformation of the contents (or as determined in these experiments, dislocation of cerebrospinal fluid) is greater than in the group of fully adult and old animals. This general finding would seem, at first glance, to be in direct opposition to one's preconceived ideas that the younger animals should have smaller coefficients of elasticity than the older. Were elasticity in the physicists' sense merely a matter of stretch or distensibility, these findings would seem to be erroneous, but the power to resist deformation appears here to be a complex function of the cerebrospinal system, compounded of vascular factors (systemic vasomotor tone, intradural vascular readjustment, etc.) as well as the inherent distensibility and collapsibility of the dural sac. And it must be emphasized that the elasticity of this latter anatomical mechanism is largely related to the method and character of the suspension of the spinal dural envelope in the vertebral canal. The possibility of a greater inward collapse of the spinal dura in animals of advancing age does not seem too remote for consideration. There is at hand no definite knowledge of the relative size of the epidural space in dogs of different ages; nor is it known whether the fibrous trabeculae of this space increase with age rather than diminish in functional importance due to increasing depositions of fat. If, as repeated inspection indicates, the volume of this epidural tissue is relatively greater in adult and old animals than in immature, the inward collapse of the spinal dural tube in old animals might be far in excess of that in young. Again, there is a strong possibility that

the relative intradural volumes of blood, of brain and spinal cord, and of cerebrospinal fluid undergo definite age-changes; here also we are confronted with impressions rather than established anatomical facts for the dog. A relative decrease in the fixed tissues of the intradural space in advancing age would increase the mobile elements relatively and therefore change the coefficient of elasticity. Such explanations of the general phenomenon of the difference in cerebrospinal elasticities in animals of different ages have much in their favor as a hypothetical consideration: they lay greater stress upon the anatomical factors than upon the purely physiological ones of vascular tone. It may be however that the more important age-change occurs in the vascular system, but such age-changes as are known physiologically in this system are those of the general systemic circulation rather than of the intradural.

The exact determination of the coefficient of elasticity of the cerebrospinal system in dynes per square centimeter permits the introduction of a definite component of elasticity into current conceptions of the degree of rigidity imposed upon the central nervous system by the bony coverings of cranium and vertebral arches. The *Monro-Kellie* hypothesis may now be restated with very definite knowledge of the elastic factor of the cranio-vertebral contents (Weed and Flexner, 1932).

SUMMARY

In dogs of different sizes, weights and ages, the relationship between the dislocation of the cerebrospinal fluid and its pressure, expressed in the fraction $\frac{dV}{dP}$, has been determined by tilting the animals from the horizontal to the two vertical positions. The total volume V of the intradural contents (cranial and spinal) has also been measured. These data have been substituted into the physical formula for the determination of the coefficient of elasticity $E = V / \frac{dV}{dP}$. The coefficient of elasticity has been found to have the following values in dogs:—immature, 4.63 to 4.52×10^5 dynes per cm.^2 ; young adult, 4.39 to 4.09×10^5 dynes per cm.^2 ; adult, 4.03 to 4.02×10^5 dynes per cm.^2 ; old, 3.84 to 3.78×10^5 dynes per cm.^2

BIBLIOGRAPHY

- WEED, L. H. 1929. Intracranial pressure in health and disease. Chap. iii, p. 25 (Assoc. Research Nerv. and Ment. Dis., vol., viii, Baltimore).
 1929. Arch. Surgery, xviii, 1049.
 WEED, L. H. AND L. B. FLEXNER. 1932. Bull. Johns Hopkins Hosp., 1, 196.
 WEED, L. H., L. B. FLEXNER AND J. H. CLARK. 1932. This Journal, c, 246.
 WEED, L. H. AND P. S. MCKIBBEN. 1919a. This Journal, xlviii, 512.
 1919b. This Journal, xlviii, 531.

OBSERVATIONS ON THE CIRCULATION IN THE FETAL ALBINO RAT

E. L. COREY

From the Physiological Laboratory of the University of Virginia Medical School

Received for publication April 18, 1932

Hartman, Squier and Tinkelpaugh (1930) have reported that the fetal heart rate of the monkey (*Macacus rhesus*) is lower than that of the mother. They consider the macaque to be exceptional in this respect. The observations of Clark (1927) indicate that in man, the cow and the dog the fetal heart rate is roughly double that of the mother. Williams (1913) states that the human fetal heart rate is between 120-140, while the average maternal rate is 70 beats per minute. In a summary of 300 cases, Corfield (1930) agrees with the findings of Williams.

The idea that the female fetal heart rate is uniformly higher than that of the male is denied by both Williams and Corfield. Observers appear to be in agreement that a high degree of variation in the fetal heart rate is present. Hartman and his co-workers state that the fetal heart rate of the monkey is "unpredictable."

The factor or factors governing the fetal heart rate remain as obscure as ever. Rech (1931a, b) in two brief papers, has brought forth evidence that no correlation exists between the blood gas content of the fetal and maternal blood. Kellogg (1930) reports very little difference in the carbon-dioxide content of the blood of very young and term fetuses. From the above, the desirability of studying cardiac activity in an extended series of fetuses throughout a considerable portion of the gestation period seemed evident.

The albino rat was selected for study because of its high fecundity and ease of handling. Although the rat fetus is small, a fairly wide experience with this animal (Corey, 1932) provided convincing evidence that it could be profitably utilized. The heart rate of 138 fetuses comprising 21 litters was ascertained. The blood velocity in 62 fetuses (13 litters) and the blood pressure of 16 fetuses (4 litters) were also determined. The fetuses measured from 8 to 39 mm. in crown-rump length, and represented the latter half of the gestation period.

The pregnant rat was lightly anesthetized with ether or amytal and placed on a water-jacketed operating table. The body wall was incised and one fold of the uterus drawn out on the laparotomy cloth. The uterus

and sac were then incised and the fetus observed. A minimum of stimulation to mother and fetus was produced. In recording the maternal heart rate, initial and final observations were made. Both palpation and direct observation of the heart were employed. Very little variation in the maternal heart rate was observed throughout the period of experimentation.

In very young fetuses it was found possible to observe the beating heart through the body wall; in the older fetuses, however, this was more difficult and all observations were checked by opening the thorax (with practically no hemorrhage) and observing the heart directly. The rates recorded by the use of both methods were found to be fairly constant, and no significant variation was observed in fetuses maintained in saline solution at 37°C. in contrast to those operated in air.

In computing the approximate fetal blood velocity, India (carbon) ink was introduced into the heart by means of a fine hypodermic needle, specially constructed. The needle was attached to a $\frac{1}{4}$ cc. syringe. The time elapsing between the admission of the ink and its appearance in the umbilical artery was recorded by use of a stop watch. Subsequent clearing of the fetuses and measurement of the vessels traversed by the ink yielded the approximate blood velocity. While the possible errors in the method are apparent, the results obtained may be considered to be comparable. Great care in manipulation was necessary, since clogging of the vessels, excessive pressure on the plunger and other factors often lead to poor results. Only in cases in which the manipulation was considered to be adequately uniform were the results tabulated.

Blood pressure determinations were made by means of a specially constructed manometer, involving the pressure of a thin rubber membrane on the umbilical cord to produce occlusion of the artery. On release of pressure, the influx of blood to the exsanguinated area was used as a criterion of the fetal systolic pressure. The results obtained are summarized in table 1. Figure 1 shows the results graphically.

The fetal heart fluctuates greatly in rate throughout the latter half of the gestation period. Three periods of greatest activity are apparent: at the 16.4, 23.3 and the 34.2 mm. stages. At term the fetal heart rate is slightly slower.¹ Considerable variation in heart rate may occur amongst members of the same litter: this difference amounted to 92 beats per minute in litter 24. There is no evidence of synchronous heart action among the members of a litter.

The average heart rate of male fetuses exceeds that of females in all litters observed, with but one exception (no. 13). Individual females may have heart rates greatly in excess of males of the same litter. No

¹ This is in agreement with the results reported by Hartman *et al.* on Macacus.

marked sex difference in heart rate is therefore apparent. The fact cannot be overlooked, however, that in all but one litter the male heart rate

TABLE 1

LITTER NUMBER	AVERAGE CROWN- RUMP LENGTH	NUMBER OF FETUSES IN UTERO	MATERNAL HEART RATE (BEATS PER MINUTE)	AVERAGE FETAL HEART RATE (BEATS PER MINUTE)	FETAL HEART RATE (EXTREMES)	AVERAGE HEART RATE (MALES)	AVERAGE HEART RATE (FEMALES)	AVERAGE BLOOD VELOCITY
	<i>mm.</i>							<i>mm. per second</i>
7	8.0	5	184	117	96-114			
11	15.3	8	248	184	168-196	185	180	
8	16.4	5	228	197	192-200	198	196	
3	18.8	9	216	159	136-184	160	158	
5	20.2	4	212	169	164-172	170	168	
22	20.9	10						11.1
10	21.8	7	252	213	192-223	214	210	
19	22.7	11	248	156	144-216	162	151	23.3
9	23.3	7	280	223	212-232	226	221	
6	24.0	5	228	185	164-192	192	180	
17	25.8	7	228	198	188-212	204	192	15.0
16	26.4	7	240	195	184-212	212	186	8.0
1	27.2	4	212	138	123-143	143	133	
23	27.3	8						4.7
21	31.7	8						10.8
14	32.6	8	256	189	176-200	190	188	13.8
12	34.2	7	264	243	224-256	246	240	
13	34.8	6	250	206	196-216	206	206	10.3
24	35.0	8	224	159	128-210	169	148	10.0
20	35.2	7						14.2
25	35.4	8	216	207	184-248	211	203	26.1
15	35.6	8	208	208	204-212	212	204	31.8
18	36.0	6	228	191	160-216	202	185	21.8
2	38.6	3	238	219	208-232	232	212	
4	39.2	5	228	185	172-196	188	180	

Total number of fetuses observed: 171.

Average blood velocity: 15 mm. per second.

Average blood pressure: 10 mm. Hg.

Average maternal heart rate: 223 beats per minute.

Average fetal heart rate: 187 beats per minute.

Average heart rate for male fetuses: 195 beats per minute.

Average heart rate for female fetuses: 187 beats per minute.

Blood pressure readings were made from litters 8, 9 and 10.

The anesthetic used on each animal has been omitted from the table since similar results were obtained with the use of either amytal or ether.

averaged somewhat higher. This finding can hardly be attributable to coincidence.

A distinct parallelism between the maternal and fetal heart rates is

apparent (see fig. 1). The fetal heart rate is observed to rise and fall, in the majority of cases, in approximate correlation with that of the mother. Vagal stimulation of the maternal heart was performed in litters 8, 9 and 10. No observable change took place in the heart rate of the fetuses *in utero*. No explanation of this maternal-fetal correlation in heart rate is offered at this time.

Checks on blood velocity and blood pressure roughly corresponded to the above observations. While the inaccuracies involved in obtaining the blood velocity are appreciated, the results may nevertheless be considered as comparable. Both blood velocity and pressure determinations parallel approximately the heart rate curve. The latter can be taken as a

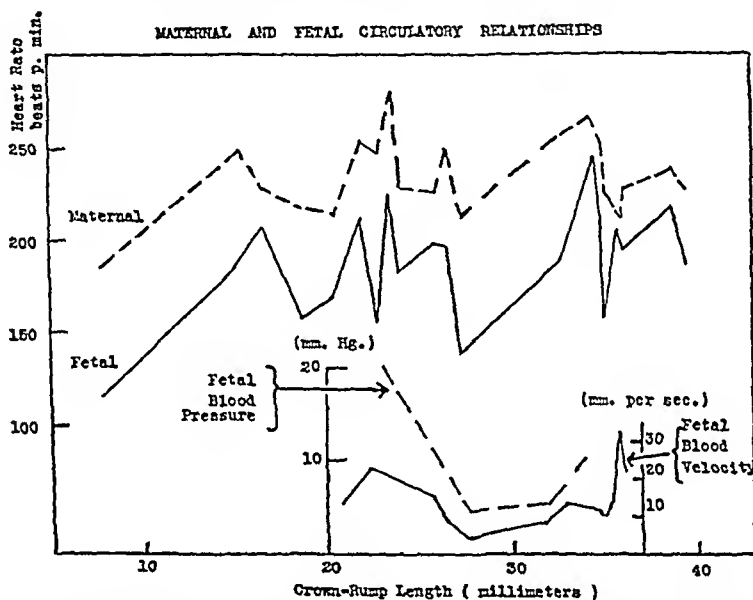


Fig. 1. Showing maternal and fetal heart rates (above); fetal blood velocity and blood pressure (inset below) during the latter half of the gestation period.

fair representation of cardiac activity throughout the latter half of the gestation period.

SUMMARY

The heart rate, blood velocity and blood pressure during the latter half of the gestation period have been determined in the albino rat fetus.

The fetal heart rate is lower than that of the mother. There is an apparent correlation between the heart rate of the mother and that of the fetuses *in utero*.

The heart rate does not increase regularly with advancing fetal development; considerable variation is observed. Three major rhythms in cardiac activity were noted during the portion of the gestation period studied.

There appeared to be a slight sex difference in heart rate in favor of the male.

Curves showing the blood velocity and blood pressure parallel approximately that representing the heart rate.

BIBLIOGRAPHY

- CLARK, A. J. 1927. Comparative physiology of the heart. Macmillan.
COREY, E. L. 1932. Journ. Exp. Zool., lxi, 1.
CORFIELD, C. R. 1930. Lancet, i, 803.
HARTMAN, C. G., R. R. SQUIER AND O. L. TINKELPAUGH. 1930. Proc. Soc. Exp. Biol. and Med., xxviii, 285.
KELLOGG, H. B. 1930. This Journal, xci, 637.
RECH, W. 1931a. Arch. f. Gynäk., cxliv, 564.
1931b. Arch. f. Gynäk., cxlvii, 82.
WILLIAMS, J. W. 1913. Obstetrics. Appleton.

THE EFFECT OF CASTRATION IN THE GUINEA PIG UPON THE SEX-MATURING POTENCY OF THE ANTERIOR PITUITARY¹

AURA EDWARD SEVERINGHAUS

*From the Department of Anatomy, College of Physicians and Surgeons Columbia
University*

Received for publication April 23, 1932

The dependence of the gonads upon the anterior pituitary for their normal development is established, and likewise the fact that gonadectomy results in striking cellular changes in the hypophysis. Engle (1929) added to an understanding of this pituitary-gonadal relationship by implanting the anterior lobes of castrated rats into immature mice and rats. He demonstrated in a series of 31 animals that the ovarian response of immature mice and rats to fresh transplants of anterior pituitary from castrated rats was far greater than that obtained with similar transplants from normal animals. These results were substantiated by Evans and Simpson (1929). Such findings are of particular interest because of the cytological changes which Addison (1917) and others had described in the pituitary of the castrate rat. These changes consist largely in an increase in the number of large basophiles, and in the appearance of vacuolated "signet ring" cells which have been called the "castration cells." Engle related the increased gonad-stimulating potency of the castrate's pituitary to the presence of these basophilic castration cells, being "led to suspect that it is the basophile cell which elaborates the gonad stimulating factor in the rat," and that the castration cells represent storage of the hormone.

An examination of the literature indicated, however, that in most of the laboratory mammals, gonadectomy results in an increase of acidophilic cells in the pituitary. Fichera (1904) first described such an acidophilic increase in the guinea pig, and he was confirmed by Kolde (1912) and others. In the light of previous experiments on the rat, where the cytological picture is in such contrast, it was of interest to test the gonad-stimulating influence of the anterior pituitary of the castrated guinea pig against that of the normal. The present paper deals with such experiments.

EXPERIMENTAL. A series of 60 guinea pigs, half males and half females, constituted the pituitary donors. Fifteen males and fifteen females were

¹ Aided by a grant from the National Research Council, Committee for Research in Problems of Sex, administered by Dr. P. E. Smith.

gonadectomized, and a similar number in each sex kept as controls. The guinea pigs were all of one inbred stock, and gonadectomy was performed at the approximate age of 30 days when their body weights ranged around 300 grams. The gonadectomized animals were sacrificed at intervals ranging from 72–205 days. Their body weights as well as those of the controls sacrificed with them were carefully recorded. In the normal females the stage in the oestral cycle was determined by daily observations and at sacrifice was recorded in the data as the number of days following the closing of the vagina.

The recipients of the transplants were littermate female white mice from remarkably uniform stock supplied through the courtesy of Dr. E. C. MacDowell of Cold Spring Harbor. Three or four littermate females constituted an experimental series in which one mouse received an intramuscular implant of a whole anterior lobe from a castrated guinea pig, one mouse a whole gland from a normal guinea pig of like sex and age, and one or two mice remained as controls. In series 8 to 11, two implants of $\frac{1}{2}$ gland on consecutive days were used in place of a single implant of a whole gland. The implants were made by the Smith method. The recipients were 20 day old mice, except in series I and II in which the mice were 22 days of age. The mice were examined on the second day following the transplant for opening of the vagina and sacrificed for autopsy on the third day following (age 23 days).

At autopsy the ovaries and the uteri were weighed and their appearance under the binocular microscope noted. All material was fixed in Bouin, sectioned at 8μ and stained in Harris hematoxylin and eosin. Engorged uteri were always drained of their fluid content before weighing to meet the legitimate criticism of Engle (1927, p. 104) that a large part of the variation in uterine weight consisted of a transitory fluid content. Several guinea pig pituitaries both of castrated and normal males and females at ages corresponding to the implant series were preserved for cytological study according to a method previously described (Severinghaus, 1932). Data are presented in composite form in table 1.

Ovarian and uterine response to transplanted pituitaries. The gonad-stimulating potency of the pituitary transplant is measured by the weight and development of the immature mouse ovaries. The greater the weight and development, the greater the amount of gonad-stimulating hormone contained in the transplanted pituitary. An analysis of the data shows that pituitaries from castrate guinea pigs are able to effect uniformly a greater growth response in the ovaries of recipient immature mice than is produced by similar implants of pituitaries of normal guinea pigs. This is true both for male and female donors, and in the females regardless of the stage in the oestrous cycle of the controls. The average weight of the ovaries following transplants of anterior lobe from castrated animals is

TABLE 1

Gonadal-stimulating action of anterior pituitary from normal and castrated guinea pigs

SERIES	NORMAL AND CASTRATED GUINEA PIG DONORS					RECIPIENT IMMATURE MICE					
	Number of animal	Sex	Days castrated	Weights		Number of animal	Treated at		Autopsy		
				At age 1 mo.: time of castra-tions	When sacri-ficed		Age	Weight	Age	Weights	
				grams	grams					Ovaries	Uterus
							days	grams		grams	grams
I	11	♀	183	331	680	34	22	7.05	25	0.0054	0.0294
	22	♀	0	320	502	33	22	7.10	25	0.0041	0.0215
						32		7.27	25	0.0029	0.0052
						35		6.85	25	0.0028	0.0067
II	12	♀	185	320	760	47	22	8.10	25	0.0067	0.0415
	20	♀	0	385	620	46	22	7.70	25	0.0078	0.0437
						45		8.80	25	0.0025	0.0091
III	15	♀	185	295	550	91539	20	10.75	23	0.0083	0.0355
	18	♀	0	285	595	91538	20	10.90	23	0.0058	0.0461
						91537		11.77	23	0.0046	0.0120
IV	16	♀	185	362	590	91542	20	9.62	23	0.0116	0.0444
	21	♀	0	358	455	91543	20	9.68	23	0.0057	0.0509
						91541		10.15	23	0.0040	0.0127
V	1	♂	186	307	590	91647	20	9.35	23	0.0074	0.0523
	28	♂	0	350	530	91545	20	9.52	23	0.0048	0.0372
						91546		9.77	23	0.0036	0.0115
						91548		8.74	23	0.0037	0.0087
VI	2	♂	205	336	740	92324	20	8.94	23	0.0118	0.0527
	30	♂	0	400	810	92323	20	9.05	23	0.0042	0.0520
						92326		8.70	23	0.0038	0.0084
						92325		9.45	23	0.0034	0.0110
VII	3	♂	205	402	810	92320	20	9.29	23	0.0079	0.0485
	29	♂	0	390	790	92322	20	9.33	23	0.0046	0.0465
						92321		9.79	23	0.0036	0.0086
						92319		9.09	23	0.0037	0.0081
VIII*	31	♂	104	350	610	92308	20	9.22	23	0.0095	0.0442
	38	♂	103	340	590		21				
	39	♂	0	?	660	92306	20	11.19	23	0.0058	0.0475
	26	♂	0	275	440		21				
						92309		11.75	23	0.0025	0.0080

* In series VIII-XI inclusive two implants of $\frac{1}{2}$ gland each were given on consecutive days.

TABLE 1—*Concluded*

SERIES	NORMAL AND CASTRATED GUINEA PIG DONORS					RECIPIENT IMMATURE MICE					
	Number of animal	Sex	Days castrated	Weights		Number of animal	Treated at		Autopsy		
				At age 1 mo.; time of castrations	When sacrificed		Age	Weight	Age	Weights	
				grams	grams					Ovaries	Uterus
							days	grams		grams	grams
IX	33	♂	104	390	600	92318	20		23	0.0110	0.0412
	7	♂	206	378	550		21				
	40	♂	0	?	540	92313	20		23	0.0056	0.0550
	9	♂	0	430	860		21				
						92315			23	0.0039	0.0135
X	48	♀	78	405	505	92412	20	10.94	23	0.0088	0.0615
	60	♀	72	408	440		21				
	50	♀	0	270	340	92411	20	10.99	23	0.0054	0.0270
	56	♀	0	405	465		21				
						92413		9.95	23	0.0038	0.0093
XI						92410		11.23	23	0.0042	0.0123
	48	♀	78	405	505	92401	20	10.39	23	0.0097	0.0482
	60	♀	72	408	440		21				
	50	♀	0	270	340	92398	20	10.98	23	0.0054	0.0520
	56	♀	0	405	465		21				
XII						92397		10.38	23	0.0040	0.0156
						92399		11.17	23	0.0051	0.0115
	8	♂	206	356	600	92402	20	9.02	23	0.0051	0.0465
	10	♂	0	385	890	92403	20	9.22	23	0.0068	0.0463
						92404		9.74	23	0.0036	0.0102
XIII**	a	♀	4½ mo.			M1379	20	9	23	0.0084	0.0433
	C111	♀	0			M1380	20	9	23	0.0032	0.0263
	C149	♀	0			M1378	20	9	23	0.0033	0.0143
XIV	b	♀	4½ mo.			M1376	20	11	23	0.0068	0.0477
	C115	♀	0			M1369	20	10	23	0.0054	0.0324
	C165	♀	0			M1377	20	12	23	0.0041	0.0300
	C107	♀	0			M1359	20	12	23	0.0036	0.0120

** Series XIII, XIV are from previously unpublished data of Smith and Engle.

0.0085 gram while that from normals is 0.0050 gram. The control non-implanted mice show a total ovarian weight of 0.0036 gram. These weight increases, while significant, are not so great as those reported by Engle for the rat.

The gross appearance of the ovaries is also of interest. Almost inva-

riably the ovaries which had been stimulated by pituitaries of castrates showed clearly visible follicles, often presenting a surface of berrylike appearance. Although small follicles were seen occasionally in ovaries in response to an implant from a normal guinea pig, not infrequently the enlarged ovary showed externally no distinct follicles.

Microscopic examination of the ovaries is more instructive. Those which have been subjected to the influence of an implanted pituitary from a castrated guinea pig present a picture of mature development. A row of fully formed follicles makes up the bulging peripheral contour of the gland, and is responsible for its gross "berry" appearance. The increased size is obviously due to this large number of matured follicles. The maturing response of the ovary to a pituitary implant from a normal guinea pig is comparable to that just described, but is a response of lesser degree. This is especially true as regards the number of follicles which have been influenced to develop; and of this smaller number, many do not depict the complete growth so characteristic of follicles acted upon by an implant from a castrate's pituitary. The ovarian responses described above are in all respects similar to those reported in the mouse by implants of pituitaries from other species (Smith and Engle).

It is of interest to note that uterine weight increases are not always proportional to the increases in ovarian weight. In several instances a slightly enlarged ovary, following a pituitary implant from a normal guinea pig, was associated with a greatly enlarged and engorged uterus. Microscopic examination of these cases showed the presence of a very few greatly enlarged follicles in an otherwise immature ovary. A similar condition was described after implants of cat pituitary (Smith and Engle, 1927, fig. 27). Obviously the introduced hormone is of such nature and amount as to influence only a few follicles which are of proper physiological state, and it does not call forth the more general growth and maturing response of follicles which larger amounts of hormone are capable of. These few follicles, however, are sufficient to cause marked changes in the uterus. The uterine response is thus the result of a few activated follicles, although the ovary as a whole may have changed very little in size, weight or development.

In series II the implant from guinea pig 20 deserves, perhaps, a word of comment. It will be noticed that the pituitary implant from the normal female has produced in this instance a slightly larger ovarian response than is shown by the littermate sister which received the implant from a castrated guinea pig. This exception to the otherwise uniform data is interesting in view of the fact that the normal donor had been observed to have irregular cycles. The uterine weight was about one-half that of normals. It is quite possible that some ovarian pathology resulted in changes in the pituitary which increased its potency. The ovaries did not appear cystic, but, unfortunately, were not preserved for microscopical study.

DISCUSSION. The rather uniform data lead unmistakably to the conclusion that the anterior pituitary of the castrated guinea pig contains more sex-maturing hormone than does the pituitary of the normal control. These results are in agreement with the findings of Smith and Engle (unpublished but here included) in a smaller series of guinea pigs. They are of particular significance because of the great cytological difference between the anterior pituitary of the castrate rat and the castrate guinea pig. In the rat, as has been pointed out, castration results in a marked increase of large basophiles and in the appearance of numerous vacuolated "castration cells" which are unmistakably modified basophiles. That these basophiles are the source of the gonad-stimulating hormone in the rat and that the castration cells represent its storage, a supposition made by Engle, seems almost irresistible. Thus the cytological findings meet all the requirements of an explanation for the increased potency of the castrate rat pituitary.

The transplantation of the castrate guinea pig pituitary has complicated the explanation. In this gland we have a similar though less marked increase in potency after gonadectomy, but no noticeable increase in the basophilic cells and a conspicuous absence of the typical "castration cell." Although the counting of a large series of animals is necessary to determine cell type proportions accurately and to detect any deviation from the normal, a survey of serial sections through the pituitaries of a few castrate guinea pigs is sufficient to confirm previous investigators that a noticeable increase in basophiles does not occur, and, so far as the writer is concerned, to leave one unwilling to deny the assertion that an actual increase of acidophiles has taken place. Such an increase could certainly not be established by routine examination of a few animals. A more detailed cytological study of the pituitary must be left for another time. What cells, then, in the anterior lobe of the castrate guinea pig are responsible for the increased amount of gonad-stimulating hormone? No answer seems convincing at this time. It becomes necessary, however, in view of these results to avoid any generalization concerning the specific cells responsible for the sex-maturing hormone. We are again faced with the necessity of admitting species differences to our interpretations. What is so obviously the case in the rat cannot possibly be applied to the guinea pig. Although these experiments do not further us directly in our attempt to localize the cells of origin of the gonad-stimulating hormone, they are valuable in reminding us again that results obtained in experiments on one species cannot forthwith be applied to all forms.

It is a pleasure to acknowledge the invaluable assistance of Dr. P. E. Smith in these studies, especially in the autopsies of the implanted mice.

SUMMARY

1. The ovarian response of immature mice following the intramuscular transplantation of one anterior pituitary from a gonadectomized guinea pig of either sex is significantly greater than the response to a similar transplant from a normal animal.

2. Although the series of transplantations is small, it indicates no sex-difference in the potency of the gland in either castrates or normals.

3. Glands from castrates of 72 days' duration and those of 206 days did not differ significantly in potency.

4. Inasmuch as the basophiles do not increase following castration in the guinea pig and the typical "castration cells" are missing, these two cell types cannot be designated respectively as the source and storage of the increased gonad-stimulating hormone.

5. These experiments compel caution in the generalization of data, and emphasize the importance of species differences in the interpretation of endocrine phenomena.

BIBLIOGRAPHY

- ADDISON, W. H. F. 1917. *Journ. Comp. Neurol.*, xviii, 441.
ENGLE, E. T. 1929. *This Journal*, lxxxviii, 101.
EVANS, H. M. AND M. E. SIMPSON. 1929. *This Journal*, lxxxix, 371.
FICHERA, G. 1905. *Arch. Ital. Biol.*, xliii, 405.
KOLDE, W. 1912. *Arch. Gynäk.*, xcviii, 505.
SEVERINGHAUS, A. E. 1932. *Anat. Record*, liii, no. 1.

THE END OF THE SPIKE POTENTIAL OF NERVE AND ITS RELATION TO THE BEGINNING OF THE AFTER-POTENTIAL

HERBERT S. GASSER AND HELEN TREDWAY GRAHAM

*From the Department of Pharmacology, Washington University School of Medicine,
St. Louis*

Received for publication April 15, 1932

The least known part of the action potential of nerve is that immediately at the end of the spike. The form of the spike has been well worked out up to the time when its potential, having fallen to a few percent of its maximum value, becomes fused with the after-potential. As the diphasic artifact causes its most disturbing distortion at this time, the region has been very resistant to further analysis. A clue which promised a solution appeared in some experiments on veratrinized nerve (9). When such a nerve was stimulated there appeared, just after the diphasic artifact, a second trough (as in fig. 3, 1 in the present paper) which in turn was followed by the after-potential, the latter not reaching its maximum until somewhat later. The conditions of the experiment were such that there was no possibility that the crest following the diphasic artifact was a delayed wave, and the only reasonable solution which presented itself was that all the negativity subsequent to the diphasic artifact was not after-potential as had been assumed, but that a portion of it belonged to the end of the spike.

The little trough in the early part of the after-potential had to have a name for reference purposes, and we gave it the purely descriptive one of N_2 , or second notch, the first notch being the diphasic artifact. When N_2 had once obtruded itself upon our attention in veratrinized nerves, a search for it in normal nerve and in nerves otherwise treated showed that it could generally be identified in the same position, though its size was such as to cause it to be easily overlooked or dismissed as an artifact. In form it was usually not a notch at all, but merely the locus of a more rapid change in the slope of the line. The constancy of the phenomenon indicated that it must have a meaning, and the experiments reported in this paper were designed to test the hypothesis that it is occasioned by the end of the spike.

That there should be in a "monophasic" lead a remnant of the spike after the apparent end of the diphasic artifact is at first sight surprising

Cessation of stimulation for half a second in a fatigued muscle results in a considerable but transient increase in both tension and action current when stimulation is resumed.

An explanation of these phenomena is offered, involving the hypothesis that under normal conditions the action current or the process underlying it determines quantitatively the amount of energy made available for the development of tension. The tension exerted by a fatigued muscle at a given frequency of stimulation is thus a resultant of the "equilibration level" of the conducting mechanism and the rate of relaxation of the individual twitches.

BIBLIOGRAPHY

- ABRIAN, E. D. AND D. W. BROKE. 1929. *Journ. Physiol.*, lxvii, 119.
- BISHOP, G. H. 1927. *This Journal*, lxxxii, 462.
- BISHOP, G. H. AND A. S. GILSON, JR. 1927. *This Journal*, lxxxii, 478.
- BOGUE, J. Y. AND R. MENDEZ. 1930. *Journ. Physiol.*, lxi, 316.
- BRISCOE, G. 1931. *Journ. Physiol.*, lxxi, 292.
- BRISCOE, G. AND W. LEYSHON. 1929. *Proc. Roy. Soc.*, cv, 259.
- BROOK, D. 1930. *Journ. Physiol.*, lxi, 306.
- COOPER, S. AND J. C. ECCLES. 1930. *Journ. Physiol.*, lxi, 377.
- FORBES, A. 1922. *Physiol. Rev.*, ii, 361.
1921. *This Journal*, lvi, 273.
- FORBES, A. AND L. H. RICE. 1929. *This Journal*, xc, 119.
- FORBES, A., H. DAVIS AND E. LAMBERT. 1930. *This Journal*, xcv, 142.
- FULTON, J. F. 1925. *This Journal*, lxxv, 235.
1925. *This Journal*, lxxv, 261.
1926. Muscular contraction and the reflex control of movement. *Baltimore*.
- GERARD, R. W. 1927. *Science*, lxvi, 495.
- KATO, G. 1924. The theory of decrementless conduction in narcotised region of nerve. Tokyo.
- LUCAS, K. 1907. *Journ. Physiol.*, xxxvi, 253.
- MATTHEWS, B. H. C. 1931. *Journ. Physiol.*, lxxi, 64.
- TSAI, C. 1931. *Journ. Physiol.*, lxxiii, 382.
- VISSCHER, M. B. AND P. W. SMITH. 1930. *This Journal*, xcv, 121.

We may emphasize again the relatively low rates of stimulation which are most effective for sustained contraction. Our results are completely in accord with those of Briscoe (1931), and we believe that our interpretation is adequate to explain all of her phenomena. The rates which we find effective for sustained contraction agree well with those directly observed in the reflex discharge governing postural tone (Adrian and Bronk, 1929).

SUMMARY

When a circulated soleus nerve-muscle preparation of a cat is stimulated for two or three minutes, the tension developed by the muscle falls from its initial value to a lower level which is then maintained with little or no further change for many minutes. The gastrocnemius muscle under these conditions sometimes does not quite maintain a steady state, and at best maintains only a smaller fraction of its initial tension than does the soleus.

The tension maintained in a muscle after three minutes of stimulation is a function, among other things, of the frequency of stimulation. If the condition of the preparation is good, the curve relating the tension exerted by the fatigued muscle to frequency shows a maximum for the soleus between 15 and 20 stimuli per second. For the gastrocnemius the corresponding frequency is between 30 and 50 per second.

The low "fatigue level" associated with high frequencies of stimulation is not due to a "Wedensky inhibition."

A muscle brought to a low "fatigue level" by continued stimulation at high frequency will partially recover if stimulated at a slower rate. The tension rises to approach that characteristic of the new rate of stimulation. On returning to the more rapid rate there is usually a transient further increase in tension, indicating that the muscle has partially recovered even while exerting the greater tension.

When the tension developed within the first second of stimulation is plotted against frequency of stimulation, the plotted curve rises with increasing frequency along an S curve up to at least 60 per second for soleus and 120 per second for gastrocnemius.

Fusion of twitches into a smooth tetanus usually occurs at a lower frequency in the fatigued than in the fresh muscle. This depends upon a slower rate of relaxation which is one factor favoring the maintenance of relatively large tensions at low rates of stimulation.

The first spike of the (diphasic) action current of the muscle after the "fatigue level" has been reached is smaller the more rapid the rate of stimulation.

If, in a fatigued preparation, the frequency of stimulation is abruptly altered, keeping it above the limit required for fusion of twitches, tension and action currents increase or decrease in parallel, approaching their new levels asymptotically.

no relation between variations in the height of the initial (electrical) deflection and in the force of contraction." It is obvious that even if the initial phase of the action current of muscle serves to initiate the chemical changes resulting ultimately in contraction, contraction might still be impaired by conditions affecting the contractile mechanism which might leave the conducting and excitatory mechanisms unaltered. The effects of calcium and of acetylcholine (Bogue and Mendez) and of water (Bishop and Gilson, 1927) we must tentatively interpret in this way. As Bogue and Mendez significantly remark "on the other hand, there is no evidence that the height of the electrical response can be reduced without affecting that of the mechanical responses."

In supposing that the action current is the mechanism which initiates and, other things being equal, determines the extent of the chemical processes resulting ultimately in the development of tension, we refer only to the "initial spike" of the muscular action current described by Bishop and Gilson (1927). The subsequent plateau which they also describe apparently depends upon the chemical or physical events in the contractile mechanism.

If our hypothesis is correct, the problems of the maintenance of tension in a muscle and of its response to various frequencies of stimulation become in part a problem of a process of "equilibration" or adaptation of the excitatory and conducting mechanism. We may expect the general principles deduced for nerve (cf. Gerard, 1927) to apply likewise to this situation. For example, deepening the ether anesthesia depresses markedly the tension developed by fatigued muscle. This is readily explained in terms of the recognized effect of ether in prolonging the refractory period of excitable tissues (Kato, 1924; Tsai, 1931).

It should be evident that the factor which prevents a muscle fatigued under rapid stimulation from developing as much tension as when fatigued under slower stimulation is not an exhaustion of the available store of fuel (e.g., glycogen) or the rate at which oxygen is supplied to it, since appropriate slowing of the rate usually causes an immediate increase in tension. Evidence has been presented to show that the effect is not due to a failure of the individual fibers to respond. The limiting factor must therefore lie in the mechanism determining the amount of energy released by each excitatory impulse. This does not mean, however, that the performance of a muscle under continued stimulation is independent of the blood flow through it. Dr. C. E. Leese is accumulating evidence on this aspect of the question, and it appears that if the blood supply is inadequate, the tension of the fatigued muscle will fall to a very low level practically independent of the frequency of stimulation. Such a relationship in no way affects our interpretation, since the tension developed by a muscle may obviously be reduced by various independent factors. Too high a frequency of stimulation is only one of these.

ished in inverse ratio to the rate of stimulation. No such diminution was observed by Bronk (1930) in excised muscle, but unfortunately his observations were confined to tetani of only 2 seconds' duration or else to a single slow rate of stimulation. Neither do Visscher and Smith's (1930) observations touch the point, as their frequencies of stimulation were so slow as to yield practically discrete twitches. Any such extreme lowering of efficiency seems unlikely, however. The diminished action currents also indicate strongly that the energy released by each impulse is reduced. This follows qualitatively from the approximately parallel variations in action current and tension, whether we regard the energy liberation as governed by the process which yields the action current, or whether the action current results from the liberation of energy for the contraction. The reduced tension resulting from more rapid stimulation is then an expression of a greater reduction in the energy liberated per impulse than can be offset by the greater frequency of the impulses.³

Such a reduction associated with rapid frequencies of response immediately suggests the familiar refractory period of nerve and muscle. It is well known that the action current of both types of tissue is reduced in the refractory period, and also that the refractory period is prolonged by continued activity. The "equilibration" of Gerard (1927) must also be considered. We have a clear case of "equilibration" in the reduction observed in the size of the action currents during the first few seconds of stimulation. They fall to a level which is lower the more rapid the rate of stimulation. Whether, as in nerve (Forbes and Rice, 1929), the further fall in action currents to the fatigue level is due to a further progress of equilibration as opposed to a lengthening of the refractory period, we have not attempted to determine. The distinction is of no consequence in the present argument. If we assume that the liberation of energy causing contraction is determined in amount, other things being equal, by the process which underlies the action current, or directly by the action current itself, we have a satisfactory explanation of the phenomena. In favor of the latter suggestion we have the familiar ability of an electric current to produce a persistent local (cathodal) contraction independent of a propagated disturbance (Lucas, 1907). It is on this basis that we are inclined to interpret the slight increments of tension yielded by an already active but fatigued muscle in response to intercurrent direct stimuli (see p. 350 above). There is also a considerable amount of evidence (Fulton, 1925a, b, 1926) that action currents and contractile tension vary *pari passu* in a variety of conditions.

This hypothesis is not necessarily refuted by the observations of Bogue and Mendez (1930) who state, in regard to cardiac muscle, that "there is

³ This statement must be modified slightly if the efficiency of the muscle is significantly reduced under these conditions.

explain the rising branch of the curve at low frequencies of stimulation. As is already well known, fusion generally occurs at a lower frequency in the fatigued than in fresh muscle.

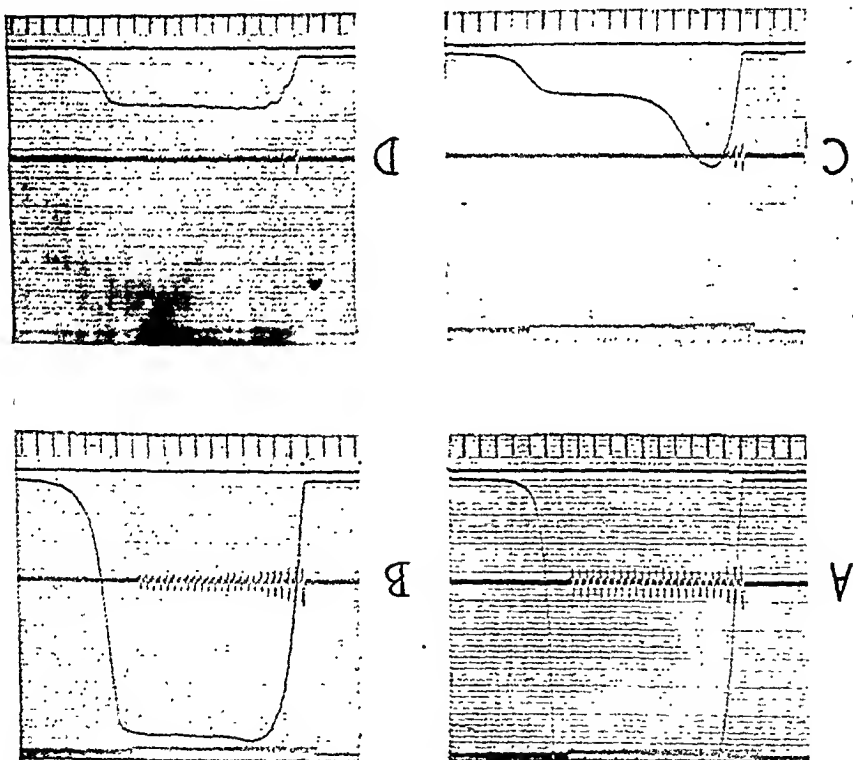


Fig. 5. December 14, 1928. Gastrocnemius muscle. Intermittent stimulation for periods of $\frac{1}{2}$ second separated by $\frac{1}{2}$ second rest.

A. Second period of stimulation. Tension record goes off film. The second recording fiber referred to in text was not in use during this experiment. B. 24th period of stimulation, showing slightly reduced tension and action currents.

C. 45th period, showing initial hump falling to a much lower plateau. Action currents during plateau are barely discernible on original film.

D. During the half second period following C frequency of stimulation was slowed from 52 to 26. This record shows the third period of stimulation at the reduced frequency. The initial hump is absent but the plateau is higher than in C. Time interval at bottom equals $\approx \frac{1}{2}$ second. Next line is line of zero tension.

A second factor must also come into play to diminish the effectiveness of rapid stimulation on fatigued muscle. It is apparent that when the frequency of stimulation surpasses a certain rate, the energy liberated per impulse must be reduced, even in the case of the fresh muscle, for otherwise the efficiency of the muscle in terms of the H/T ratio would be dimin-

fibers; but this possibility seems remote. In fact, the very regular gradual increase in the size of the action currents of the muscle strongly suggests that we are dealing rather with a process of "equilibration" in the muscle itself.

A few observations have been made in which the activity of the muscle has been broken up into alternate periods of half a second of contraction and half a second of rest. The frequencies of stimulation, as in most of the experiments on sustained contraction, were 30 and 15 per second for the soleus and 50 and 25 for the gastrocnemius. The results were essentially similar for the two muscles, and a description of one will suffice. The gastrocnemius, stimulated in this fashion, responds with a series of tetanic contractions which at first continue to increase in tension throughout the entire half-second of stimulation. In the course of about half a minute the contractions each show a level plateau, the maximum being reached in about a fifth of a second. Then, by degrees, more rapidly with the more rapid rate of stimulation, the tension begins to fall off during the latter part of each tetanus, until ultimately we find a brisk initial twitch, rising to perhaps 50 per cent of the tension developed by the fresh muscle, followed by a rather abrupt drop to a definite plateau which is maintained for the remainder of the half-second of activity. This plateau is lower and the initial twitch is higher for the rapid stimulation than for the slower. The level of these plateaus becomes essentially constant for a given frequency and obviously corresponds to the "fatigue level" attained with continuous stimulation. If the frequency of stimulation be changed there is a gradual shift to the picture characteristic of the new rate of stimulation, the twitch and the plateau being modified in opposite directions.

The action currents in such an experiment correspond closely in size to the tension developed by the muscle. When the fatigued state is reached, the action currents at the beginning of each brief tetanus are large, comparing favorably with those recorded from the fresh muscle. They rapidly shrink to a much smaller size, corresponding to the low plateau which terminates each tetanus. These findings are illustrated in figure 5. The interesting features of this experiment are the rapid recovery which the muscle makes during each half-second of rest, and the inability of the muscle to maintain this recovered state. The benefits of the rest are intense but fleeting. Half a dozen impulses suffice to bring the muscle back to the fatigue level. The situation recalls that described by Matthews (1931) in the recovery from adaptation of the sensory end-organ in the muscle of a frog.

DISCUSSION. The curve relating the tension developed in brief tetani to the frequency of stimulation can probably be explained entirely as the result of more or less perfect fusion of the individual twitches. For the tension maintained at the "fatigue level" the same principle serves to

each impulse and the impulses would be large enough to excite the muscle. This interpretation would imply that the low tension developed under rapid stimulation is due to the blocking of impulses in some of the neuro-myal junctions and the relaxation of the corresponding muscle fibers. The maintenance of the tension which does persist might be explained on the basis of alternation of activity among various motor units. In any case the "Wedensky" interpretation implies that a large proportion of the muscle fibers become completely inactive.

This explanation is inadequate in the present case, although we cannot rule it out absolutely as a minor contributing factor. We sought experimentally for inactive fibers in the muscle by applying supramaximal induction shocks directly to the muscle during its tetanic contraction. For this purpose the galvanometer leads were transferred to an inductorium by means of a double-throw switch. Since the contraction of the muscle is virtually isometric, the resulting twitch in any idle fibers should add almost quantitatively to the preëxisting tension and be easily detected. The result of this test was negative. In a typical experiment a soleus muscle was fatigued by stimulation at the rate of 30 per second until the tension was 35 per cent of its initial value of 2250 grams. Slowing the stimulation to 15 per second resulted in an increase of tension to 71 per cent of the initial maximum. This transition is illustrated in figure 3. Again speeding up the stimulation, the tension fell eventually to 20 per cent. Intercurrent direct stimuli now caused increases of tension of 77 grams, as compared with 530 grams developed in response to the same stimulation 3 seconds after the cessation of the tetanic stimulation. Therefore at most only 14.5 per cent of the fibers could have been idle during the tetanic stimulation. This will not explain the diminution in tension under the high frequency of stimulation, or even the difference in tension exhibited by the two frequencies. Probably even fewer fibers are idle than is suggested by the above figures, since there is an alternative explanation for the increment of tension in response to direct stimulation which will be considered below.

Further evidence against the Wedensky phenomenon is found in the behavior of the action currents and tension immediately after the slowing of the rate of stimulation. The shift of frequencies is absolutely abrupt, as shown in figure 3. The resulting change in action currents and tension is gradual. If we were dealing with a Wedensky inhibition, however, the first impulse to arrive after the longer interval should be much larger than those at the higher frequency and should at once excite a large number of idle fibers. We should expect a sudden increase in both tension and action currents. The only alternative is to assume that a still further increase in the size of the nerve impulses due to "equilibration" (Forbes and Rice, 1929), is necessary to bring them to a strength adequate to excite the idle

the largest and 4 the smallest of the group. The change from one pattern to another is always gradual and never abrupt. In the majority of experiments, however, alternation of any sort is either very slight, absent, or endures for only a small part of the time, and is associated with the higher frequencies of stimulation.

The passage of alternate impulses through a region of partial local narcosis (or a fatigued neuromyal junction) is a familiar phenomenon (cf. Tsai, 1931), and the alternation may also arise at the stimulating electrodes (cf. Forbes and Rice, 1929), either because a rising threshold causes the make shocks to become ineffective in some fibers or because of anodal blocking. We do not believe that these latter effects were significant in our experiments because the stimulus was set well above maximal for make shocks at the beginning of the experiment, and, in nearly every case in which the test was made, increases and decreases of about 50 per cent in the strength of the stimulus caused no visible change in either tension or action currents, even in a fatigued preparation. We therefore ascribe the alternation to fatigue of the neuromyal junction.² The phenomenon is significant only as showing that some fibers in the muscle were not responding to every stimulus at the more rapid rates of stimulation, but rather were responding at only half the frequency. This in no way serves to explain the major phenomenon which is the diminution in tension which results from increasing the frequency of stimulation. On the contrary the effect would be to obscure this phenomenon. In fact we believe that the second maximum which sometimes appears in the tension-frequency curve for fatigued muscle at relatively high frequencies may be due in large part to alternation which results in some of the muscle fibers responding at a frequency only one-half that of the stimuli applied to the nerve. These fibers stimulated at a lower frequency would be expected to develop relatively more tension than those which are responding to every stimulus. It is probably more than a coincidence that, when present, the second maximum usually appears at double the frequency of the main one.

Diminution of activity associated with a higher frequency of stimulation immediately suggests the phenomenon of "Wedensky inhibition" (cf. Forbes, 1922) as a possible explanation. In this case we would suppose that, as fatigue develops, the relative refractory period of the nerve or neuromyal junction becomes so prolonged that the tissue does not have time to recover completely between successive impulses at the higher frequency. The impulses will therefore be subnormal in size and may become so small as to fail to excite the muscle fiber. At the lower frequency of stimulation, however, recovery would be almost or quite complete after

² The alternation can hardly have been dependent upon irregular spacing of the stimuli, since no differences in the intervals between the action currents can be detected, and alternation was not an invariable phenomenon.

since there is no question of fusion, as in the case of twitches, to complicate the case. Measurements of the action currents recorded about one second after the beginning of stimulation show only a slight depression at the highest frequencies employed. In the case of fatigued muscle, however, a depression with increasing frequency is very marked, as may be seen in figure 1. The very extreme depression, however, may be due in large measure to interference of the second phase of one action current with the first phase of the next.

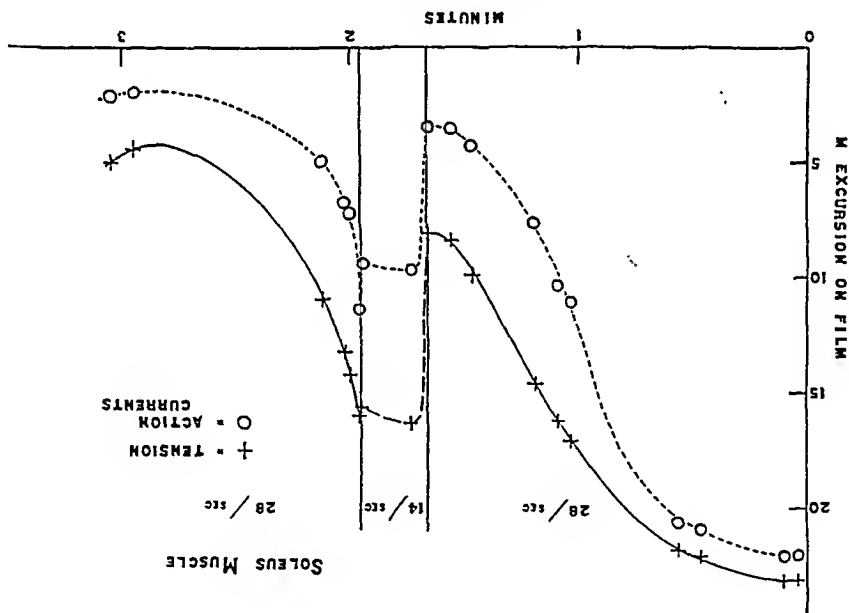


Fig. 4. March 26, 1929. Soleus muscle. Tension and action currents both measured arbitrarily in millimeters excursion on the film and plotted against duration of continuous stimulation. Vertical lines indicate moments of transition from frequency of 28 per second to 14 per second and reverse. Note the increase in both tension and action current on slowing the frequency and also the transient further increase on again stimulating at the higher frequency. Stimulation by rotary interrupter and induction coil. Make and break shocks effective.

In some experiments the action currents show a well-marked alternation in size, when the stimuli are relatively rapid and the muscle is fatigued. This indicates that some fibers are not responding to every stimulus. When the alternation appears it is usually quite regular and not by any means a random variation in the height of successive excursions. The most usual situation is that every second action current is five or ten per cent smaller than the ones preceding and following it. Differences of as much as twenty per cent are rare. Sometimes the pattern is more complicated although still regular, sequences of four action currents showing successive relative sizes in the order 1, 4, 2, 3 or 1, 3, 2, 4, where 1 indicates

at a high frequency of stimulation it can partially recover at a lower frequency *while maintaining a greater tension*. It should be noted that there is here no question of an incomplete tetanus in either case. The paradox can be achieved with rapid enough frequencies to give complete fusion throughout the experiment.

Action currents of the active muscle, recorded simultaneously with the contraction, offer a valuable clue to the solution of this paradox and to the nature of the maximum in the curve relating tension to frequency. Unfortunately the action currents as recorded are diphasic, since it was desired not to injure the muscle to render them monophasic, lest we interfere with the maintenance of tension. Changes in the height of the excursions of

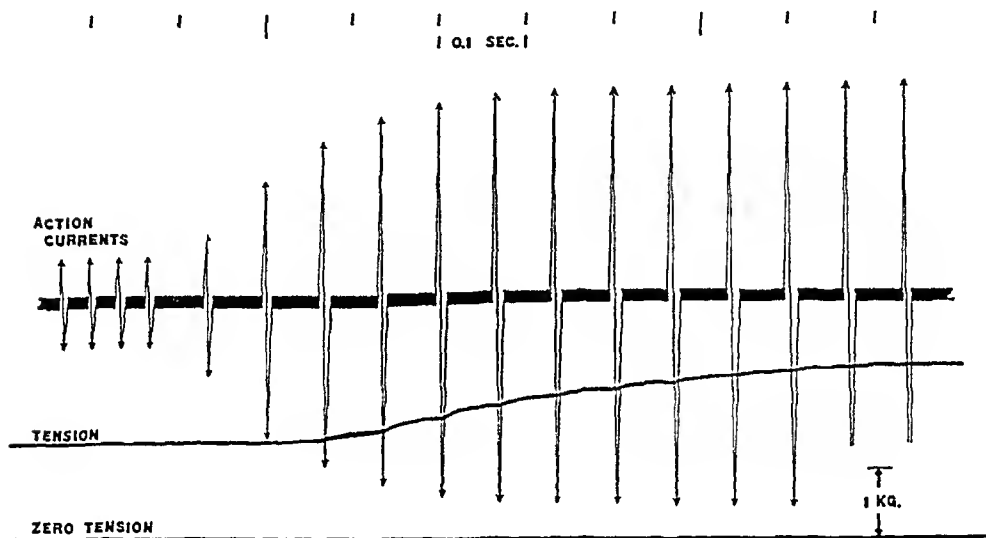


Fig. 3. March 26, 1929. Soleus muscle. Increase in tension and in height of action currents resulting from change in frequency of stimulation from 30 to 15 per second, redrawn to scale from original film. Stimulation at 30 per second had been in progress for 1 minute 40 seconds.

the galvanometer may therefore be due to some extent to a slowing of the rate of electrical change and an alteration of the time relations and relative strengths of the two phases; and indeed the action currents in the fatigued muscle show signs of all of these changes, which are already well recognized as characteristic of fatigue. There remains, however, a gross change in the size of the action currents, running closely parallel to the changes in tension, which it is impossible to explain by these minor alterations. We wish to emphasize this general correlation, which is illustrated in figure 4, rather than any detailed quantitative comparison.

The relationship between frequency of stimulation and the height of the first phase of the action current differs from the curve for tension,

soleus muscle has first attained the level characteristic of 30 stimuli per second, and that the frequency of stimulation has then been reduced to 15 per second, and that the tension has increased to the new level. If, within a short time after the slowing of stimulation, when the new level has not quite or has only just been reached, the frequency is again increased, then the tension will drop again promptly to the first fatigue level. This fall in tension is not as abrupt as the relaxation when stimulation is discontinued entirely. In fact, if the muscle has been stimulated for some

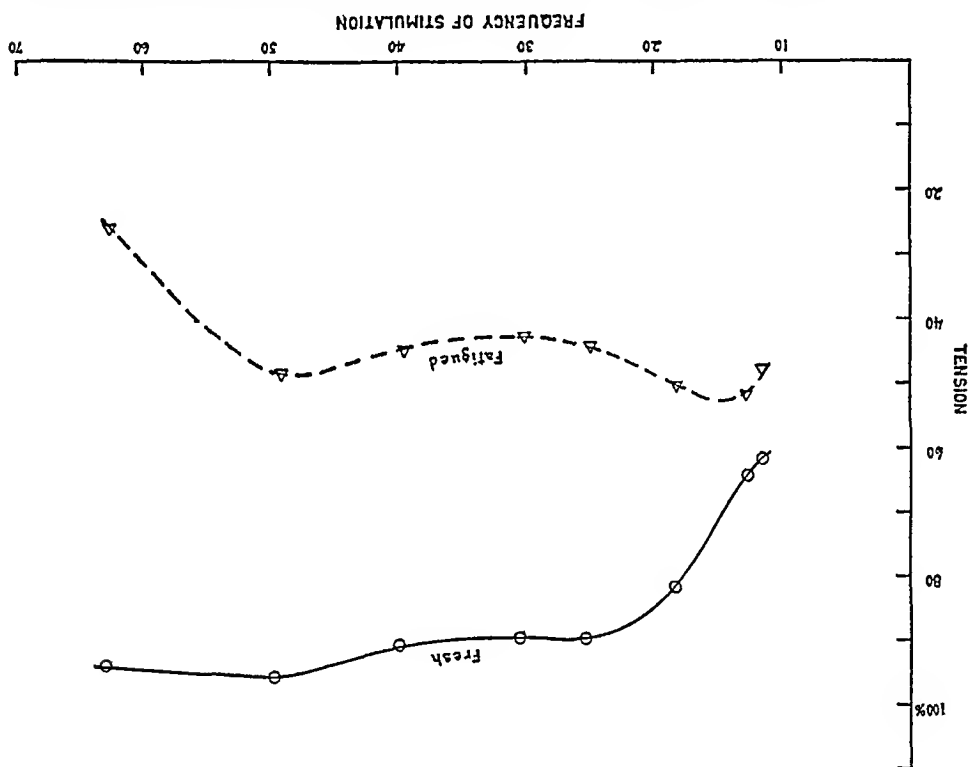


Fig. 2. November 16, 1931. Soleus muscle. Tension expressed as percentage of tension developed during test periods of 2 seconds' duration, stimulating at 62 per second. Observations made in random order. Blood pressure range from 110 to 145 mm. Hg. Other details as for figure 1.

time, half a minute or more, at the lower frequency, there is usually a transient rise in tension on speeding up the stimulation. This rise is only transient, being succeeded by the usual fall, and the greatest tension attained is never as great as that developed by a fresh muscle stimulated at the rapid rate in question. Even so, the rise in tension shows that the muscle after a period of stimulation at low frequency is nearer to the fresh state than when it is in the fatigued state characteristic of the high frequency. This leads to the paradoxical conclusion that after a muscle has been fatigued

which has been inactive for some time. Our highest figure for the soleus under these conditions is 175 σ at 30°C. This same muscle, after many alternate periods of activity and rest, showed a value of 100 σ for the first contraction after a minute's rest, and of 50 σ for one of a series of partially fused twitches a minute after the beginning of stimulation at 9 per second. We have many records of twitches of fatigued soleus muscles which are scarcely 40 σ in duration from the first electrical disturbance to the maximum of tension. The duration of twitches of the gastrocnemius may shorten from 45 σ to 15 σ . Cooper and Eccles have already called attention to this phenomenon, but we also observe that in a fatigued muscle the duration is brief even though the total tension is slight. Therefore the higher total tension invoked by Cooper and Eccles in partial explanation of the phenomenon can hardly play a part in this case. The briefer time to maximum found in fatigue is not surprising, however, since if a smaller amount of energy is liberated by an impulse it should presumably expend itself in a briefer time.

We have already mentioned the two phases of relaxation, the first of which usually merges imperceptibly into the second, but sometimes, if it be slow and linear, gives way abruptly and forms a clear "nose" or "angle." The second phase is relatively constant but the first varies considerably in duration, rate of fall, and degree of curvature. The usual effect of fatigue is to slow this process and yield a more perfectly fused tetanus. If the circulation is good, however, the effect of activity may be actually to accelerate the first phase of relaxation. This is usually followed by a subsequent slowing.

The net result of these variations, which apparently depend upon a variety of factors which we have not undertaken to identify and which may aid or oppose one another in tending to produce a smooth tetanus, is to make the relation between fatigue level and frequency very uncertain except in its broad qualitative features. The difference between similar observations on a fresh muscle and on the same muscle after several periods of work, illustrated in figure 1, is an excellent example of this. Inspection of the original records shows the lower branch of the curve is due in this case to imperfect fusion consequent on a shortening of contraction time and acceleration of the first phase of relaxation.

The data for figures 1 and 2 were obtained by separate tests at each of the various frequencies. In other experiments the frequency was altered abruptly without allowing a cessation of activity. In such experiments if a soleus muscle which has been brought to its fatigue level with a rapid rate of stimulation, say 30 per second, is now stimulated at 15 per second, the resulting tension almost immediately begins to increase, as shown in figure 3. The tension rises to the value characteristic of the new rate of stimulation, reaching it in approximately half a minute. Suppose now that a

minutes of rest, recovery is far from complete, so that it is impossible to make many observations on the same muscle under comparable conditions. In view of the greater expenditure of energy in this muscle this situation is not surprising, and it is significant that the muscle specialized for phasic activity often fails to maintain a steady state during continuous activity while the postural muscle (soleus) rarely fails.

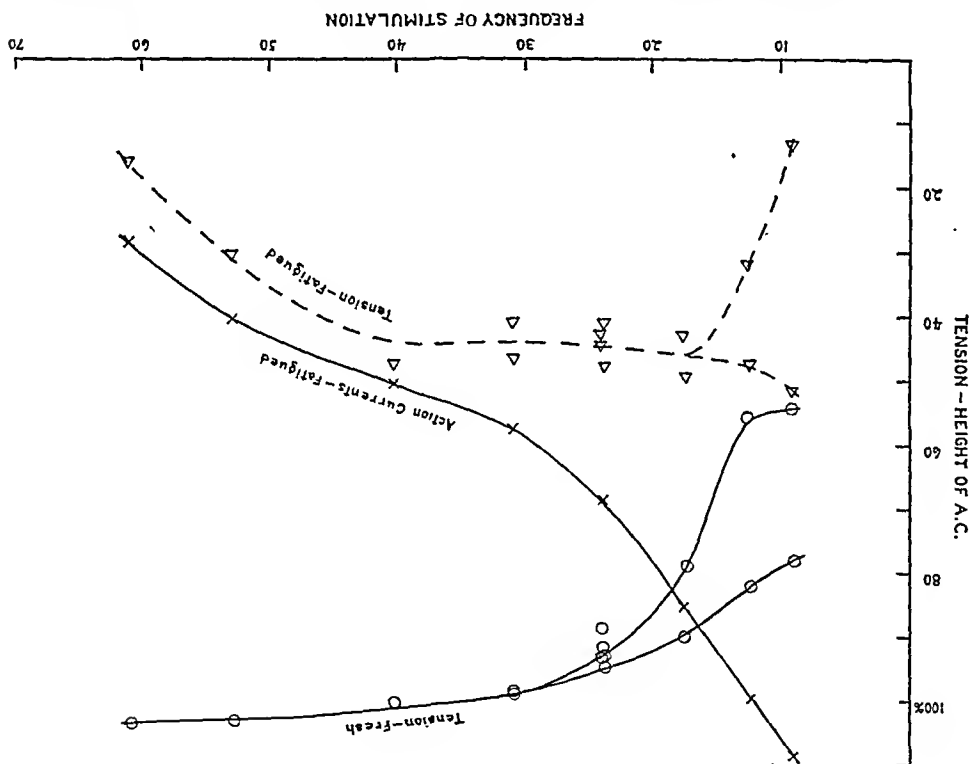


Fig. 1. December 4, 1931. Soleus muscle. Stimulation by helium tube stimulator. Frequency of stimulation expressed in shocks per second. Isometric tension developed by muscle expressed as percentage of the tension developed in a test stimulation at frequency of 31 per second of the height of the first action current of the series. Observations made in regular sequence beginning with slowest frequency, going to highest and returning to low frequencies again. The well sustained tensions at very low frequencies represent the performances at the beginning of the series. Other details in text.

Among the variables which render performance of fatigued muscle difficult of precise definition, whether in the gastrocnemius or the soleus, are changes in the time values characterizing the individual muscular twitch. The time to maximum of tension has generally been implicitly accepted as relatively constant for a given type of muscle, but it may vary over a wide range. This "contraction time" seems to be greatest in a fresh preparation

If, instead of a rate of 30 per second, we employ stimuli at 15 per second, the tension rises less rapidly and does not reach such a high maximum. At first the contraction is not perfectly fused but shows clearly the individual humps corresponding to the separate impulses. As stimulation is continued these humps smooth out and gradually become completely or almost completely fused. The tension eventually falls somewhat, but to a lesser degree than with the more rapid rate of stimulation. Thus with the slower stimulation the tension is at first lower and later higher than it is with the more rapid rate. This relation of tension to frequency of stimulation is expressed graphically in figure 1, in which are plotted the maximal tensions and also the fatigue levels attained by a single muscle at various frequencies of stimulation. This is the most complete and best controlled of several experiments of this type. The blood pressure rose gradually from 87 to 104 during the first seven observations and remained within ± 2 mm. of this value for the remainder of the experiment except for a sudden transient fall to 84 during the observation at the frequency of 61 per second. The muscle was stimulated for periods of three minutes and allowed to rest for five. The frequency was systematically increased by steps from 9 per second to 40 per second, then decreased in reverse order, then alternated between 24 per second and the high frequencies of 52 and 61 per second. The results show lower fatigue levels at frequencies above 40 per second. This figure is unusually high for the point above which tension becomes less, but this may be due in part to the excellent general condition and circulation of this particular preparation. Another type of curve sometimes met with is shown in figure 2. The relationship of fatigue level to frequency of stimulation shows a clear maximum at 15 or 20 per second and a second less clearly defined maximum at about 40 per second. The genesis of these maxima and also the reason for the divergence of the curves in figure 1 at low frequencies of stimulation will be considered below.

An attempt was made to establish a similar curve for the gastrocnemius, but it became evident that there were so many possible variables in the situation that it would be difficult to obtain a "typical" curve and that any absolute values would be of little significance. However, by piecing together observations from a number of experiments we may say with confidence that qualitatively the same relationships hold as for the soleus and that the "speed" of the muscle is about double that of soleus, (i.e., the fatigue sets in in half the time, the optimal rate of stimulation and the rate necessary for fusion, are both about double, etc.). The gastrocnemius does not lend itself as readily as the soleus to this type of experiment. It is more difficult to attain an even approximately "steady" state or fatigue "level," for the tension usually continues to fall slowly but steadily. Furthermore, after two minutes of steady stimulation, followed by five

sponse and the beginning of rapid relaxation is about 65 σ at the end of a minute of stimulation. This means that a frequency of 16 per second may give a tetanus which is not grossly unsteady. The corresponding figures for the soleus are 100 to 120 σ and 9 or 10 per second. Most of our experiments have been performed with frequencies of approximately 50 and 25 per second for the gastrocnemius and approximately 30 and 15 with the soleus. The lower frequency in each case will usually give a smooth or very nearly smooth tetanus in a muscle which has been in activity for a minute or so, while with the higher rate we obtained a contraction from the fresh muscle nearly as powerful as those elicited with much higher rates of stimulation.

Preliminary experiments showed that the exact length at which the muscle was called upon to work had little effect upon the behavior or condition of the muscle, provided it was not stretched beyond the initial length to which it may be extended by flexing the ankle to the maximum degree possible in the intact animal. Between this limit as one extreme and the point at which the muscle is extended only one or two millimeters beyond the point just sufficient to take up all slack in the tendon, the total tension developed in a *tetanus* is nearly independent of the initial length of the muscle. There is a slight increase with increasing initial length, but in one experiment with the soleus this was less than a 20 per cent increase on going from the point of just perceptible initial tension to well beyond the physiological limit. In the case of the gastrocnemius the increased tension due to increasing the initial length is somewhat greater than in the soleus. If either muscle has once been extended beyond the physiological limit, irreversible or slowly reversible changes take place which make it impossible to reproduce quantitatively results obtained previously with a lesser degree of extension. We therefore performed most of our experiments at an initial extension sufficient to stretch the muscle about a millimeter beyond the point of slackness. This corresponds roughly to the condition at semi-flexion of the ankle joint in the intact animal.

SUSTAINED ACTIVITY IN RELATION TO FREQUENCY OF STIMULATION. If the circulated soleus muscle be stimulated (indirectly) at a rate of 30 per second, the tension will continue to increase for several seconds at the beginning of stimulation. This tension is then maintained for a short time but at the end of a minute the tension has fallen perceptibly. It continues to fall, at first more rapidly and then more slowly, for the next two minutes or more, finally reaching a level of perhaps 1100 grams as compared with an original maximum of 2600 grams. The absolute values, of course, vary with the size and condition of the animal. This low level of tension will then be maintained without further decrease, if the blood pressure is good, for half an hour or more. The tension associated with this approximately steady state we shall speak of as the "fatigue level."

improving the fixation of our preparations and their physiological condition in efforts to obtain the angle, the plateau associated with it, and also a smoothly fused tetanus at low frequencies of stimulation. From time to time we obtained humps in the early phases of relaxation, but the most pronounced angle which we ever obtained was described in our protocols at that time as being only "fair." We did not discover that the angle was due to friction in the bearing of the myograph lever, as Cooper and Eccles have demonstrated (1930), but our own experience leads us to concur absolutely in this interpretation of the phenomenon in the case of the single twitch in fresh muscle. We now use a myograph whose bearing is a knife edge rocking in a shallow rounded depression instead of the original cylindrical rod resting in a V notch. Even with this myograph, however, the end of a tetanus is often marked by a relatively sudden change in the rate of fall of tension. At the end of two or three minutes of stimulation at relatively low frequency this discontinuity is most marked and forms a definite "angle" in Fulton's original sense. Under these circumstances it cannot be due to friction in the myograph, since it is obtained with a frictionless lever which shows no angle on a single twitch. Furthermore the tension is definitely falling previous to the angle, the curve being slightly concave downwards or practically linear. This slow fall then merges more or less abruptly into the familiar logarithmic curve which is concave upwards. In the case of single twitches or very brief tetani the first phase of slow relaxation is poorly developed and nothing appears but a point of inflection in the curve of relaxation.

In concordance with the work of Cooper and Eccles, we found that the tension developed in a brief tetanus in both soleus and gastrocnemius is greater the greater the frequency of stimulation up to approximately 40 or 50 per second for the soleus and to somewhere above 100 persecond for the gastrocnemius. Frequencies of stimulation of this order of magnitude are required to obtain a smooth tetanus with a fresh muscle. In all respects in which we have performed similar experiments, our results agree qualitatively and quantitatively with the descriptions and values given by these workers, except that in our experience the time to the maximum of tension of a single twitch is even more variable than their data indicate. We did not pursue these aspects of the question in great detail, however, as our interest centered in the behavior of the muscles during sustained contractions, and it soon became evident that a muscle which had been in activity for a minute or more would usually give a smoothly fused contraction at a rate of stimulation far lower than that which was required at the beginning of the experiment.

The formation of a smooth tetanus at low frequencies depends upon the development of a slow initial phase of relaxation as described above. In the case of the gastrocnemius the interval between the last electrical re-

through the shaft of the tibia at the junction of the lower and middle thirds, and the other through the lower end of the femur for fixation of the gastrocnemius or through the head of the tibia for fixation of the soleus. These drills were rigidly clamped to a heavy animal table of adjustable height.

The sciatic nerve was severed in the popliteal space, and the branch serving the soleus and gastrocnemius muscles identified. When the activity of the soleus muscle alone was to be recorded, the nerve was usually dissected free where it passes through the gastrocnemius muscles, and all branches to the latter severed. The tendons of the two muscles were always dissected free from one another and also from the other muscles contributing to the Achilles tendon. This provided essentially a circulated neuro-muscular preparation, free from reflex activity, having fairly stable blood pressure and remaining in good condition for many hours. In the more recent experiments the blood pressure was recorded during the myographic observations. Evaporation from the surface of the muscle was minimized by smearing it freely with petrolatum or wrapping it loosely with a layer of moist and then a layer of dry cotton. Its temperature remained at approximately 30°C., or a little higher, throughout the experiment. Action currents were led off to the string galvanometer through agar electrodes ending in cotton wicks, one of which was stitched lightly to the surface of the lower third of the muscle and the other to its tendon. This yielded simple diphasic action currents. In later experiments fine silver needle electrodes were substituted for the agar electrodes.

For stimulation, shielded electrodes of the Sherringtonian type, or, in later experiments, tubular electrodes (cf. Forbes, Davis and Lambert, 1930) were applied to the motor nerve. An induction coil of the type described by Bishop (1927), with an iron core, furnished shocks strong enough to be definitely supramaximal on both make and break. These were delivered to the nerve at frequencies controlled by the rotary interrupter described by Forbes (1921). In the early experiments the frequency was varied by changing the speed of the interrupter, but in later experiments an instantaneous doubling or halving of the frequency was obtained by switching the connections to a different pair of brushes while the interrupter continued to run at a steady speed. When intermittent tetanic stimulation was required, the stimuli were periodically short-circuited by means of a mercury switch operated by a metronome. Still more recently a neon tube or helium tube stimulator closely similar to the one described by Briscoe and Leyshon (1929) has been employed. At the beginning of these experiments we were surprised and somewhat disconcerted to find ourselves unable to demonstrate a clean-cut angle at the termination of the single muscular twitch as described by Fulton (1926). We devoted considerable time to

FATIGUE IN SKELETAL MUSCLE IN RELATION TO THE FREQUENCY OF STIMULATION¹

HALLOWELL DAVIS AND PAULINE A. DAVIS

From the Laboratories of Physiology of Harvard Medical School

Received for publication April 4, 1932

The following experiments were begun in the fall of 1928 as a control study for an investigation as to the nature of the muscular asthenia associated with the complete removal of the adrenal glands. In view of the complexities disclosed by the control experiments the latter problem has been indefinitely postponed, but the behavior of circulated skeletal muscle of the cat in response to stimulation at varying frequencies exhibits points of independent and timely interest. In particular it seems to afford an explanation for certain phenomena recently described by Briscoe (1931) in the course of her search for a mode of stimulation which would reproduce normal postural activity.

APPARATUS AND METHODS. Most of the experiments were performed upon the circulated gastrocnemius or soleus muscles of the cat, and in most cases ether anesthesia was employed, until we became aware of the differences which might be introduced by too deep an anesthesia. Subsequent experiments performed with decerebrate preparations yielded results indistinguishable from those obtained from animals under light ether anesthesia. Muscular contractions were recorded isometrically, or nearly so, by means of a torsion myograph of the type described by Fulton (1926). The torsion member is a piece of tool steel 75 mm. long and 6 by 1.5 mm. cross section. The recording arm of the myograph projects into the eyepiece of a Cambridge string galvanometer. The latter is connected to the muscle through suitable electrodes, so that we obtain a simultaneous record of the mechanical and the electrical events in the muscle. The magnification of the shortening of the muscle was approximately 40 \times in experiments on soleus, and 150 \times in the case of the gastrocnemius. The recording arm was provided with two vertical fibers, so spaced that when the shadow of the first was about to leave the edge of the photographic record the second appeared on the opposite edge, thereby doubling the effective width of the recording surface.

Fixation of the leg of the cat was obtained by two drills, one passing

¹ A preliminary report of these experiments appeared in *This Journal*, xciii, June 1930.

SUMMARY AND CONCLUSIONS

1. Histamine has been isolated from acid extracts of the pyloric mucosa as a sulphate and pterate. The method is described.
2. Vaso-depressor and secretory assays on all fractions from the original acid extract to the final product of histamine crystals and mother liquor yield strong, if not conclusive, evidence that histamine is the sole secretory excitant active subcutaneously in acid extracts of the pyloric mucosa and that histamine was not produced by the chemical procedures employed.
3. Histamine (0.5-1.0 mgm.), pilocarpine (5-10 mgm.) and iso-pilocarpine (10 mgm.) are the only imidazoles out of ten tried that stimulate gastric secretion. Methylene histamine (5-10 mgm.) does not stimulate gastric secretion.
4. A threshold dose of a gastric secretory excitant is present in 1 or more liters of urine which is to be expected on the basis of the histamine content of urine (Best and McHenry, 1930).
5. Histaminase destroys the gastric secretory effectiveness of "gastrin" solutions.
6. Histamine adsorbed to Lloyd's reagent maintains its vaso-depressor and secretory properties.

BIBLIOGRAPHY

- ABEL AND KUBODA. 1919. *Journ. Pharm. Exper. Therap.*, xxiii, 243.
 BEST AND MCHENRY. 1930. *Journ. Physiol.*, lxvii, 256.
 BARGER AND DALE. 1911. *Journ. Physiol.*, xli, 499.
 BURGESS AND IVY. 1930. *Proc. Soc. Exper. Biol. and Med.*, xxviii, 115.
 EDKINS. 1906. *Journ. Physiol.*, xxxiv, 133.
 IVY. 1930. *Physiol. Reviews*, x, 282.
 1931. *Kosmos*, iv, 80.
 IVY AND FARRELL. 1925. *This Journal*, lxxiv, 639.
 IVY AND JAVOIS. 1924. *This Journal*, lxxi, 604.
 KOCH, LUCKHARDT AND KEETON. 1920. *This Journal*, lli, 508.
 KOSKOWSKI. 1925. *This Journal*, lxxv, 640.
 KOSKOWSKI AND KUBIKOWSKI. 1929. *Compt. rend. soc. Biol.*, c, 292, 1240, 1243.
 LIM, LOO AND LIO. 1927. *Chinese Journ. Physiol.*, i, 51.
 NECHLES AND LIM. 1928. *Chinese Journ. Physiol.*, ii, 415.
 POPIELSKI. 1920. *Pflüger's Arch.*, clxxviii, 214, 327.
 SACKS, IVY, BURGESS AND VANDOLAH. 1931. *Proc. Soc. Exper. Biol. and Med.*, xxviii, 941.

of Koskowski and Kubikowski (1929) indicate that the histamine content of the blood is increased during the gastric digestion of meat.

Two other questions that must be considered are: Is histamine the sole active agent of acid extracts of the pyloric mucosa? And, might not the chemical procedures used have produced histamine from a closely allied imidazole? Koch, Luckhardt and Keeton, as a result of their chemical studies stated that histamine and "gastrin" may later be found to be identical or closely related. We adhered to this belief until we had actually obtained histamine crystals from a number of extracts and had checked the vaso-depressor and secretory effects of all fractions. One reason why we believed them not to be identical was that histamine in proper concentration is precipitated uniformly by picric and picrolonic acid, but "gastrin" is not precipitated from certain "gastrin" solutions by these precipitants. If histamine is added to a "gastrin" solution, picrolonic acid precipitates the added histamine (Koch, Luckhardt and Keeton, 1920). But picric (Koch, Luckhardt and Keeton) and flavianic acids precipitate the added histamine but poorly. We confirm Koch, Luckhardt and Keeton in regard to the action of these precipitants. Some interfering substance is evidently present which increases the solubility of histamine picrate and prevents it from being precipitated without interfering with its biological action. We believe that the biological evidence showing that the ratio of vaso-depressor to secretory activity is practically constant in all fractions from the original extract to the final step yielding crystals and mother liquor, is strong, if not conclusive, evidence indicating that histamine is the sole active secretory agent of acid pyloric extracts and that histamine was not produced by the chemical procedures employed.

Although histaminase abolishes the secretory and vaso-depressor effect of acid extracts of the pyloric mucosa, this does not prove that the active principle is histamine, since it has not been shown that histaminase acts on histamine exclusively. However, since histamine is the most potent vaso-depressant and gastric secretory excitant of all known imidazoles and of all other known substances, the inactivation of "gastrin" by histaminase constitutes strong presumptive evidence that histamine and "gastrin" are identical.

The evidence at hand, which appears to us to be complete, shows that histamine is the sole gastric secretory excitant in dilute acid extracts of the pyloric mucosa and that histamine may be the gastric hormone. *It has not been proved that histamine is the gastric hormone; neither has it been proved that there is a gastric hormone.* The final answer to these questions awaits the chemical isolation and identification of the active substances in the blood or in dialysates and the extraction of the principle or principles from the mucosa of the gastro-intestinal tract.

explains the widespread distribution of "gastrin bodies" or "gastric secretins."

However, the rather ubiquitous distribution of histamine does not necessarily discredit the probability that histamine is the gastric hormone, since it is well known that the admixture of a little vaso-dilating agent to vaso-depressor free secretin augments the pancreatic secretory response, that local vaso-dilatation is favorable for secretion in general, and that histamine may be injected intravenously at such a rate as to obtain gastric secretory stimulation without a general vaso-depression (Ivy and Javols, 1924; confirmed by Ivy and Kim, unpublished). As a matter of fact, histamine if produced in physiological amounts by the pyloric mucosa during digestion, may be viewed as not only being a specific hormone augmenting and facilitating the formation of gastric juice, but also as being a "general digestive hormone" augmenting the formation of pancreatic juice, bile, and succus entericus (Koskowsky and Ivy, 1925) and increasing the motor activity of the gastro-intestinal tract.

This view is supported by the observation of Best and McHenry (1930) on the distribution of histamine and histaminase in the tissues of the body. They find that the stomach and liver are the only organs (the thyroid has not been examined for histaminase) examined in the body which contain relatively large quantities of histamine which do not also contain histaminase. The enzyme is absent from the stomach and practically absent from the liver. From their work we can say that it is easily possible for the pyloric mucosa to liberate enough histamine into the portal blood and then into the general circulation to stimulate the production of acid by the gastric glands and to exercise a generally synergistic action on the digestive process.

The crucial question that arises in regard to the preceding discussion is: "Does histamine get into the blood during digestion?" Neeches and Lim (1928) obtained from the portal blood by the method of vivi-dialysis some agent that unquestionably stimulated gastric secretion on subcutaneous or intraperitoneal injection. They determined the blood pressure effect when they tested the dialysates for the presence of a pancreatic excitant and found that they stimulated the pancreas, causing but little depressor effect. The reports of these investigators are not entirely clear on the vaso-depressor activity of their portal dialysates. We have also made dialysates and have extracted "fed" and "unfed" blood. We find a vaso-depressor agent that is not an inorganic salt. But the results of our chemical and biological studies are as yet not sufficiently clear cut to warrant a definite statement. Hence the question asked above, we believe, cannot be answered with the evidence at hand. However it should be pointed out that Best and McHenry report that a small quantity of histamine is present in the blood (0.4 mgm. per kgm.) at all times. Further, the observations

material obtained from one liter of urine produced a slight augmentation of gastric secretion (table 1); thus a threshold dose was present. This agrees with the work of Best and McHenry (1930) who report on the basis of vaso-depressor assay, 0.2 mgm. of histamine per kilogram of urine.

On the vaso-depressor effect of the hydrous aluminum silicate-histamine compound. Since it is possible that histamine may be bound with some inert substance that would prevent it from being precipitated with picric, picrolonic and flavianic acid in certain solutions, but still permit it to exercise its vaso-depressor and secretory effects, we decided to treat some histamine with Lloyd's reagent and determine its vaso-depressor effect. It was found that histamine when absorbed to Lloyd's reagent still possessed its vaso-depressor and secretory properties, but to a somewhat less degree.

TABLE 1

Showing the presence of a gastric secretory excitant in human urine post-cebum active on subcutaneous injection

Urine extract 30.0 cc. equivalent to 1 liter of human urine.

PROCEDURE	TIME	VOLUME	FREE HCl	TOTAL HCl	OUTPUT HCl
	minutes	cc.	per cent	per cent	mgm.
Control.....	60	2.5	0.1824	0.3554	8.885
Urine extract 30.0 cc., subcutaneous injection					
Post-injection.....	60	4.0	0.3007	0.3646	14.534
	60	3.5	0.1459	0.2280	7.980

DISCUSSION. The literature on the question of the existence of a hormone mechanism for gastric secretion has been reviewed by one of us (Ivy, 1930, 1931); hence, it will not be referred to in detail here. It is sufficient to state that although it has been established that a humoral mechanism is concerned in gastric secretion, the nature of the humoral agent or agents has not been determined. The humoral agent may be either a hormone, or secretagogues, absorbed from the lumen of the bowel, or both.

The active principle of dilute acid extracts of the pyloric mucosa has been regarded as the gastric hormone "gastrin," since its discovery by Edkins (1906). However, a gastric secretory excitant has been found in extracts of many animal tissues and plants, especially in such tissues as duodenal mucosa, liver, pancreas, and thyroid (Koch et al., 1920; Ivy, 1930). The fact that all such tissue extracts on intravenous injection caused a decided fall in blood pressure led Popielski (1920) to refer to "all the secretory excitants" extractable from tissues as vaso-dilators and to regard them as non-specific. The widespread occurrence of histamine

other and with the intensity of the Pauly reaction. This must be interpreted as good evidence that histamine is the sole excitant of gastric secretion present in the extracts (active subcutaneously). The other possibility, that there is another substance present with the same ratio of vaso-depressor and secretory activities as histamine, is rather remote. *Histaminase destroys "gastrin."* The second method of showing that histamine or a very closely allied imidazole is the only excitant of gastric secretion present in the original extract, made use of the histamine-inactivating enzyme of Best and McHenry (1930). A quantity of the original acid alcohol extract of the mucosa was evaporated to remove the alcohol, taken up in water, brought to pH 7 with sodium hydroxide, and incubated with histaminase powder made from the kidney under toluene at 37° for 24 hours. The quantity of histaminase used was twice that necessary for the amount of histamine shown to be present by blood pressure assay. After the incubation, the solution was filtered, evaporated to remove toluene, and the entire quantity injected subcutaneously into a Pavlov pouch dog. Although, on the basis of previous gastric secretory assay, the solution had originally contained ten times the effective dose of histamine, there was no response by the pouch. The usual dose of histamine (0.5 mgm. of the phosphate) injected into the same dog two hours later, gave the customary response. Thus we can say from this experiment (which was repeated ten times with the same results) that the active secretory agent in extracts of the pyloric mucosa can be inactivated by histaminase, which shows that the active principle is histamine or a very closely related imidazole.

Specificity of histamine. A variety of other imidazoles have been tested for gastric secretory activity, with negative results except in the case of pilocarpine and isopilocarpine (Sacks, unpublished). Koch, Keeton and Luckhardt (1920) used histidine, methylimidazole and hydroxymethylimidazole; Burgess and Ivy (1930) used imidazole, imidazolealdehyde, imidazole propionic acid, and imidazole lactic acid in 5 mgm. doses; all yielded negative results. Methylene histamine made by the action of formaldehyde on histamine, kindly furnished by Doctors Kendall and Gebauer, was also found to be ineffective in doses of 5 to 10 mgm. Pilocarpine and isopilocarpine stimulate salivary and pancreatic secretion and comparatively large doses are required to stimulate gastric secretion. Hence histamine may be said to be the only known imidazole which is a "specific" gastric secretory stimulant.

Is a gastric secretory excitant present in urine? As imidazoles are known to be present in urine, we investigated to ascertain whether a gastric secretory excitant could be obtained from that source. We collected the urine of men that was formed following the ingestion of a meal and subjected it to treatment with Lloyd's reagent. In one experiment out of three, the

superior to amyl alcohol in that the chloroform extracts less impurity. The amount of activity destroyed varies. We also found that "semi-pure gastrin solutions" failed to yield an active picrate or picrolonate as does histamine. But more purified solutions sometimes yield a picrate. For example, on one occasion 1200 doses of "gastrin" obtained by chloroform extraction were precipitated with silver nitrate and barium hydroxide, the silver being removed by hydrogen sulphide and the barium by sulphuric acid. The solution assayed 500 doses of "gastrin." The solution was then concentrated to a small volume and saturated sodium picrate solution added in excess. On standing, a yellow crystalline precipitate was obtained which was filtered off on a Hirsch funnel and recrystallized twice from water. One hundred and fifty-five milligrams of crystals were obtained and 1 mgm. gave a good gastric response. The melting point was 217–231°; mixed melting point with known histamine dipicrate was 217–225°. No crystals were obtained from hot alcohol, but on evaporation of the alcohol and taking up the residue in hot water, crystals again formed having a melting point of 221–224°. On blood pressure assay the crystals reacted as histamine picrate. It should be added that on several occasions we were able to obtain histamine crystals from intestinal mucosa by the methods of Barger and Dale (1911) and Abel and Kubota (1919), but were unable to obtain histamine crystals by applying these methods to pyloric mucosa.

Histamine, the sole gastric stimulant present in the extract? Since the yield of crystalline histamine is rather small, it became necessary to ascertain if histamine is the only substance present in the original extract which stimulates gastric secretion, when administered subcutaneously. This was done in two ways: first, by comparing the intensity of the Pauly reaction, the vaso-depressor action, and the gastric secretory stimulation of the solutions at each step of the extraction process, with the same properties of pure histamine, and second, by using histaminase (1930). The original alcoholic extract gives a more intense Pauly reaction than would be expected from the physiologic actions, but the secretory and vaso-depressor activities were in the same ratio as in all subsequent steps. (Vandolah has found that thoracic duct lymph after feeding gives a good Pauly, but does not depress blood pressure or stimulate gastric secretion.) The Pauly reaction is not an accurate index of vaso-depressor or "gastrin" activity under all conditions. We use the term "ratio" because when "impure gastrin" and histamine having equal secretory effects are given intravenously, the depressor effect of histamine is greater. The solution left after the histamine is adsorbed on the Lloyd's reagent still gives a Pauly reaction, but has no vaso-depressor or gastric secretory effects. However, solutions at the subsequent stages of the process gave vaso-depressor and gastric secretory responses which ran parallel with each

thoroughly and the Lloyd's reagent then allowed to settle. The supernatant fluid was siphoned off and discarded. The Lloyd's reagent was then sucked dry at the pump and washed with aqueous 1 per cent sulphuric acid. It was then extracted at room temperature with 3 per cent aqueous ammonia. The ammoniacal extract was filtered by suction, evaporated to a small volume, made slightly alkaline with sodium hydroxide solution and mixed with $1\frac{1}{2}$ times its weight of anhydrous sodium carbonate. On standing overnight this became a hard cake. This cake was powdered in a mortar and extracted with chloroform in a continuous extraction device. The extraction was continued until fresh extracts no longer gave a Fauly reaction.

The combined chloroform extracts were filtered to remove some dark gummy material and then extracted with a small amount of water. The aqueous extract was filtered and boiled to remove chloroform and volatile bases. The solution was then made just acid with nitric acid and silver nitrate in slight excess was added. The precipitate which formed was separated by centrifugalization, and discarded. The mother liquor was then made neutral with barium hydroxide solution, when a small amount of dark brown precipitate formed. This too was removed by centrifugalization and discarded. To the remaining solution barium hydroxide was added in excess. This precipitated the histamine as a silver salt. The precipitate was washed free of barium suspended in water, and dilute HCl added in slight excess. This converted the histamine-silver into the soluble histamine hydrochloride and insoluble silver chloride. The latter was removed by filtration and the filtrate evaporated to a small volume. On standing overnight, a small amount of crystalline material separated from the solution. This was dissolved in 95 per cent alcohol (twenty volumes) and concentrated sulphuric acid added drop by drop, to maximum precipitation. The needles of histamine sulphate were separated by filtration and washed with alcohol and ether.

The histamine was identified by conversion into the diplicate. The rhombic yellow leaflets obtained melted at 230–232°C. When mixed with diplicate from known histamine, the melting point was not depressed. This method has been repeatedly applied successfully to different original extracts.

During the early part of our work we repeated the chemical studies of Koch, Luckardt and Keeton (1920) and confirmed them in every particular. They found that chloroform failed to extract "gastin" from an aqueous alkaline solution. This is true when chloroform is shaken with an alkaline solution of "gastin." However, chloroform will extract the active principle when the latter is mixed with anhydrous sodium carbonate and the dry, powdered mixture subjected to continuous extraction with chloroform. Although the yield is only about 20 per cent, chloroform is

HISTAMINE AS THE HORMONE FOR GASTRIC SECRETION

J. SACKS, A. C. IVY, J. P. BURGESS AND J. E. VANDOLAH¹

*From the Department of Physiology and Pharmacology, Northwestern University
Medical School*

Received for publication April 13, 1932

Since Edkins (1906) proposed the "gastric" theory of gastric secretion, there has accumulated considerable evidence indicating the existence of such a hormone mechanism. The existence of a humoral mechanism has been definitely established by the transplantation experiments of Ivy and Farrell (1925), Lim, Loo and Liu (1927), and by vivi-dialysis experiments of Necheles and Lim (1928). Koch, Luckhardt and Keeton (1920) have attacked the problem of the isolation of the active principle of extracts of pyloric mucosa. Although they did not succeed in isolating the active substance, they did discover a number of its chemical properties and stated that histamine and gastrin "may later be found to be identical or closely related."

We (Sacks, Ivy, Burgess and Vandolah, 1931) have been able to isolate histamine as a sulphate from the pyloric mucosa of the hog under conditions which preclude the possibility that it is present as a result of putrefactive changes. We have obtained other evidence showing that histamine is the only gastric secretory excitant present in extracts of the pyloric mucosa which is active subcutaneously. Our evidence indicates either that histamine is the gastric hormone, or if not, there is no gastric hormone, or the gastric hormone has never been extracted from pyloric mucosa.

Isolation of histamine. The washed pyloric mucosa of the hog, obtained as it came down the chute at the slaughter house within thirty minutes after the animal was killed, was immediately subjected to extraction with 1 per cent sulphuric acid in 80 per cent alcohol. The extraction was allowed to continue overnight, the acid liquid siphoned off, and replaced by a fresh portion. In all, three extractions were made. To the alcoholic solutions, sufficient activated charcoal was added to remove pigment and suspended material. It was found that under these conditions the activated charcoal (Mallinckrodt) did not adsorb any of the active secretory or vaso-depressor substance. After separating the charcoal by filtration, Lloyd's reagent (hydrous aluminum silicate, Lilly) was added to the clear filtrate in the proportion of 1 gram per 100 cc. The mixture was shaken

¹ Josiah Macy, Jr., Foundation Fellow.

have described a period of depression having the same duration and the same behavior with respect to temperature as the relatively refractory phase.

SUMMARY

The form of the axon spike potential has been followed to a point closer to its end than in previous determinations.

The potential of the falling phase drops below 5 per cent of that obtaining at the crest in about 3 crest times. In this region the rate of decline shows a sharp decrease so that the phase ends along a slowly decaying curve and becomes indistinguishable at 20 to 25 crest times.

When the potassium method is used for making the nerve monophasic the potential recorded is found to be much freer of diphasicity than when the indifferent lead is produced by heat coagulation. The form of the monophasic spike obtained with the potassium method is shown.

New evidence is given that in the ordinary "monophasic" lead the first phase outlasts the apparent position of the diphasic artifact. The wave which this causes in the records is labelled 7.

During at least 85 to 90 per cent of the visible duration of the spike potential the nerve shows signs of the production of another potential very different in its general behavior, the after-potential.

The after-potential has a rising phase. Like the total duration this rising phase lasts for very variable times, according to the condition of the nerve. Its crest may occur at 3.0 to 4.0 σ (estimated) at the lower limit up to 60 σ or more at the upper (temperature range 20–25°C., comparative value of spike crest-time 0.3 σ).

Cooling causes a prolongation of the rising phase of the after-potential.

BIBLIOGRAPHY

- (1) ADRIAN. 1921. *Journ. Physiol.*, Iv, 193.
- (2) AMBERSON, PARFART AND SANDERS. 1931. *This Journal*, xcvii, 154.
- (3) BISHOP. 1927. *This Journal*, lxxxii, 462.
- (4) BISHOP. 1931. *This Journal*, xcvi, 504.
- (5) ERLANGER AND BLAIR. 1931. *This Journal*, xcix, 108.
- (6) GASSER. 1931. *This Journal*, xcvi, 254.
- (7) GASSER AND ERLANGER. 1930. *This Journal*, xciv, 247.
- (8) GRAHAM. 1931. *Journ. Pharm. Therap.*, xlii, 269.
- (9) GRAHAM AND GASSER. 1931. *Journ. Pharm. Therap.*, xliii, 163.
- (10) LEVIN. 1927. *Journ. Physiol.*, lxiii, 113.

liquid chain potentials as assumed by Levin for the after-potential; or are we, in view of the striking differences in the qualities of the two potentials, to regard the spike as due to a transient interruption of the surface film according to the inherent supposition of the classical theory and to grant that the after-potential only has a chemical basis? Both views can be defended and a decision between them is impossible.

The tail on the spike calls for a reconsideration of the relation of the relatively refractory period to the spike. The fact that the nerve is absolutely refractory until the spike potential has almost subsided (Adrian), taken with the apparent absence of potential during the relatively refractory period, leads to the conclusion that there is no relation between the refractory period and the spike. Now we find that the spike potential is easily long enough to last throughout the relatively refractory period. Even so, there is still no very great parallelism between the course of return of irritability and the restoration of the normal potential, but the discrepancy finds a possible explanation in the recent observation by Bishop (1931) that the nerve fails to conduct when the resting potential is depressed 2 to 3 millivolts by various means. Irritability would not be expected to return at all until the depression of the resting potential by the action wave is less than this amount (absolutely refractory period), and the rise of irritability to normal would be simultaneous with the restoration of this final small amount of potential (relatively refractory period). Quantitative studies in this direction are needed.

Gasser and Erlanger observed that in cold frog nerve the supernormal phase disappeared and the relatively refractory period lasted as long as the action potential. On the basis of this observation they made the suggestion that in this state all the potential might belong to the spike. This surmise with respect to the nature of the potential has been confirmed in the present series of experiments by observing the course of the two potentials over a series of temperatures, as the after-potential disappeared and the spike approached its pure form in the limit. Taken together the two observations support the possibility of correlation between the spike and refractory period.

No complete correlation is possible, however, as the refractory period is known to be modified under various abnormal conditions which are not known to change the spike form. Part of the variation may be associated with the after-potential. In its later course the latter is associated with the supernormal phase; and, since it can be demonstrated to exist as early as the portion of the spike at which the absolutely refractory period ends, it might be expected to alter nerve irritability during continuance of the spike. Another factor which may alter the relatively refractory period is one which occurs without any potential sign. Following a subthreshold cathode shock, and therefore in the absence of a spike, Erlanger and Blair

be formed over a sufficient time interval to show augmentation of potential through accumulation, or they might have to be metabolized to some potential-producing intermediate, an oxidation product, perhaps, since as first shown by Amberson the after-potential is greatly depressed in asphyxiated nerves. It may be however that oxygen is necessary not for such an oxidation of catabolites, but rather for the maintenance of a state of the nerve in which the catabolites can produce a potential.

The explanation of the interval of rising after-potential on the basis of a diffusion time is insufficient, as the amount of change is too great to be accounted for on the basis of the temperature coefficient of the rate of diffusion in aqueous systems. (Because of the tendency of the after-potential to change independently of temperature, no attempt has been made to determine the coefficient. Its order of magnitude is often found to be as large as or larger than the one holding for the spike.)

The second and third suggestions concerning the period of rising after-potential postulate underlying chemical processes and therefore demand a positive temperature coefficient, but chemical hypotheses have met a difficulty in the fact that the temperature coefficient of the after-potential duration is negative. The latter however gives an incomplete picture of the behavior of the after-potential as a whole, for the development of the potential occurs more slowly when the nerve is cooled (fig. 7). Thus, as far as the genesis of the after-potential is concerned, the modification by temperature is compatible with a chemical hypothesis, for the necessary prolongation of the time of the production of secondary catabolites in cooled nerve is found. The shorter total duration however would have to be explained on another basis. A slower rate of production of metabolites might lead to a smaller accumulation, as their removal by diffusion would have a lower temperature coefficient and would therefore be relatively more effective at lower temperatures. Furthermore the surface film may be more stable. Fewer molecules would move into and out of the surface in unit time and the surface would be less susceptible to modification by addition of foreign molecules. Thus the potential would be less altered by a given concentration of a molecular species, and the concentration might fall below the threshold of potential production earlier in spite of a slower restoration to the normal resting chemical composition.

A problem whose solution is more remote is the nature of the origin of the two potentials. In addition to the previously known factors in this problem we now have the condition that the potentials occur simultaneously and without any parallelism, one may be rising while the other is falling. There is to be explained not the nerve potential but the nerve potentials. Are we to assume two chemical processes, one causing a high short-lived potential and the other a low prolonged one, both through the action of metabolites altering the constitution of the surface film or setting up

With curve B of figure 6 is included another earlier record, A, from the same nerve made when the lead was less diphasic. It identifies the T wave with unusual clearness and supports the interpretation given to the small deviation labelled T in curve B. Curve C in the same figure is a typical monophasic spike ending. The maximum amount that could be subtracted from curves A and B would be a fraction of the spike potential of such magnitude that the end of the spike would pass through the T crests. The remainders from such subtractions are drawn into the figure. Somewhere between these lines and the actual records must lie the truth of the matter. The result is a potential starting near the beginning of the nerve response and increasing to a maximum at about 7σ .

As a final illustration we may consider a purely monophasic ending from a fresh nerve in which the after-potential was small and in which the action

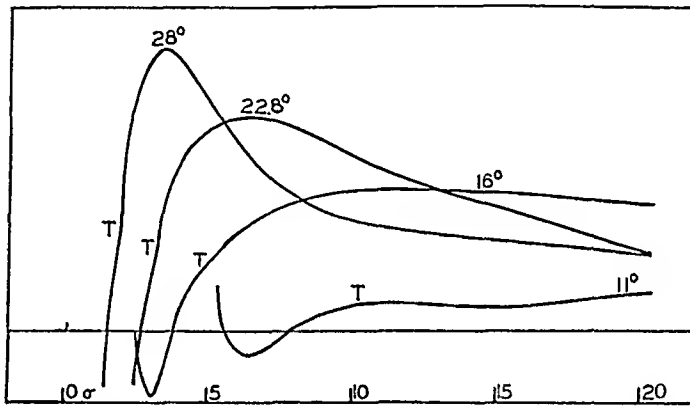


Fig. 7. After-potentials from the same nerve at various temperatures. 3/8/32. As the spike crests corresponding to these after-potentials do not have the same height the magnitudes of the after-potentials are not comparable.

potential manifested an eventless decrementation to zero (fig. 5B). When this curve is corrected for the minimum spike value as measured on the same nerve, the after-potential appears to run through a maximum under the end of the spike (5C). This is the other extreme from the condition obtaining in stimulated veratrinized nerves, and it is apparent that the crests of the after-potential may occur, according to the conditions, all the way from 3 or 4σ to 60σ or more (reference value of crest time 0.3σ).

DISCUSSION. The observations which have been described in the preceding sections necessitate a restatement of several problems in nerve physiology.

The period of rising after-potential preceding the terminal decremental curve suggests that antecedent to the period of dissipation of the source of potential there must be a period of a different nature. Catabolites might have to diffuse over a finite distance before exerting their effect; they might

ture of the wave is downward. It is only later, in the part of the curve not included in the figure, that the curve takes on a typical decremental form. Therefore, the course of the potential is not explainable on the basis of a single chemical process. What the after-potential amounts to before the spike ends is not directly visible though it might be estimated by subtracting from the potential the mean value of the end of the spike which was derived in the first section. The result would obviously be a curve of the form assumed in the parallel reconstruction, figure 2D; but the beginning of the after-potential may be obtained with less calculation in another manner.

The early part of the after-potential is revealed in purer form with parallelly diphasic leads, that is, leads in which the nerve has been heated under

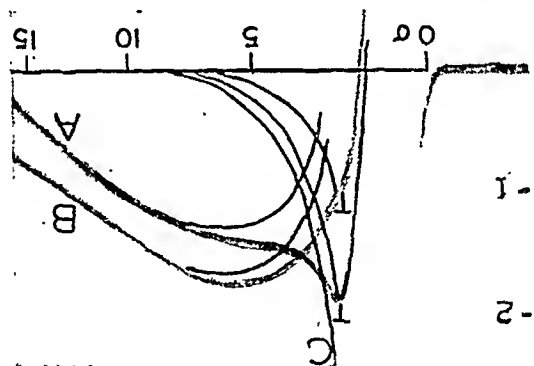


Fig. 6. Crest of the after-potential. Records of fresh nerves made with the lead at the stimulating cathode, 1/6/32, 22.2°C. Ordinates give per cent of crest height. A and B are two records, manifesting different degrees of diphasicity, printed from two films (retouched) superimposed. C is the form of the end of the spike taken from the inset in figure 1 (curve marked with circles).

the distal electrode for the purpose of obtaining a monophasic response without complete attainment of the desired result. A form frequently yielded by this type of experiment is shown in figures 3, 5 and 6B. The after-potential is recorded with a crest. Now, is this crest an artifact? The idea immediately suggests itself that the crest may be produced by the diphasicity. In this connection the T wave of the spike stands us in good stead in reaching a decision. When the T wave can be identified, we know that throughout its duration, in order to get the true after-potential picture a value must be *subtracted*, not added. The amount to be subtracted is something less than the value that the monophasic spike would have at this time; but whether one use the full value of the monophasic spike or any value less than this down to zero, the result of the correction is in the latter case to leave the form as recorded and in the former to accentuate the rising phase.

7 crest times and 0.5 per cent at 11 crest times. The potential becomes indeterminate at about 20 crest times.

In the determination of the spike duration temporal dispersion on conduction was controlled according to the usual methods, but the recent experiments of Erlanger and Blair indicate the possibility of dispersion from another cause. When a threshold induction shock is used for the stimulus a delay of about one crest time occurs in the setting up of the response under the stimulating cathode. The latency is less than this for stronger shocks but as long as the stimulus is submaximal there must be some fibers responding just at threshold and these might prolong the axon potential by a time which is the difference between the latencies of the most irritable fibers and of those responding at threshold. This prolongation would amount, however, only to about five per cent of the duration measured.

The start of the after-potential. By comparing the action potential endings at different temperatures it was previously shown (6) that as soon as three crest times had elapsed after the start of the action potential, the after-potential could be identified as such. This has been amply confirmed in a much simpler way in the present research; the part of the action potential which visibly changes form with alterations of the magnitude of the after-potential begins at the diphasic artifact. Comparison of records 1 and 2 in figure 3 gives a not particularly good illustration of this change.

About the form of the start of the after-potential little has been known. If the after-potential be due to catabolic products as has been proposed by Levin, the simplest inference would be that it would start at a maximum and proceed along a decremental curve as the products are removed by chemical change or diffusion. The first intimation that such is not the case was found in nerves treated with veratrin or with calcium-rich Ringer's solution. If these nerves are subjected to repeated stimulation they may show after-potentials rising in value for 50 sigmas or more (9). In general changes in tissue activity under the action of drugs are of degree rather than kind; they are exaggerations or suppressions of processes normally taking place. On this ground the expectation would be that normal nerves, too, would show after-potentials starting out at less than maximum. The evidence verifying this prediction is set forth in the following paragraphs.

One of the many forms of after-potential observed experimentally reveals by mere inspection that the after-potential does not decrease logarithmically throughout its course. This form is illustrated in figure 3, C. It is an axon potential obtained with a lead from a stimulating cathode on a nerve recently killed at the distal lead and having no visible diphasicity. At the start the shape of the wave is obviously due to the decremental end of the spike, but when this no longer dominates the picture the curva-

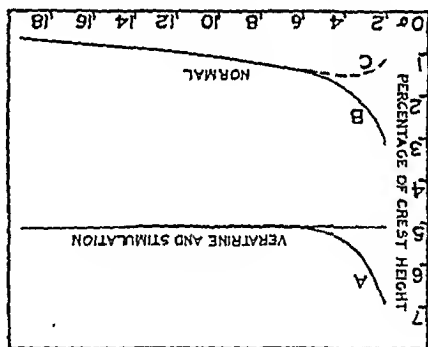
of the spike (fig. 5). The spike is thus lost to view on a plateau formed by the after-potential. In this combination the spike size can be estimated if the reasonable assumption be made that an extrapolation backward of the after-potential as a straight line parallel to the X axis should leave above the line a potential *at least* as great as that belonging to the spike, since from all that is known about the start of the after-potential any deviation from horizontal would be downward.

Experimentally it proved very difficult to get the right combination and attempts to do so resulted in numerous failures because the after-potentials obtained were either rising or falling. Three successful experiments are plotted as points in the inset in figure 1. The experiments were performed at 22.2 to 25°C., and in one case the crest time was measured and found to be 0.32σ. All three experiments must have had very similar crest times, and the monophasic spike calculated to 0.3σ as previously described is therefore comparable with these veratrinized nerve spike endings and is plotted as a line in the inset.

Considering all the elements entering into the two methods of evaluating the potential the agreement is very good. The deviation which occurs is in the reverse direction from the one which does not follow that this is a product of the methods; too few data are available for a conclusion on this point to be drawn. The action potential recorded in figure 1 has a particularly low-valued terminal portion and a comparison of it with the spike endings calculated by the other method leads to the conclusion that it can have very little after-potential in its complete position. It therefore affords the closest approximation to the complete course of the monophasic spike at present available.

Because the spike form is so greatly affected by temperature its dimensions can best be designated in terms of the crest time. The early fall of the spike potential is so rapid that it drops below 5 per cent of the crest value at about 3 crest times. At this point there is a rather sharp bend in the curve and the decline becomes more gradual. This is the point which has previously been taken quite arbitrarily as an index to the spike duration. Granted that the spike of figure 1 gives the order of magnitude of the late part of the spike, 1.0 per cent of the crest potential is left at about

Fig. 5. A, ending of spike on flat veratrin after-potential. B, normal action potential. Both records from the same nerve, made monophasic with KCl, 1/6/32. Lead from stimulating cathode. C, residual potential after subtracting the spike value measured on A.



Needless to say, temporal dispersion must be eliminated either by leading from the stimulating cathode or by using small responses with only a few millimeters of conduction. A statistical check of the experiments for the time to N_2 involved the use of many in which the crest time had not been measured; therefore only those experiments were included which were performed in the temperature range from 22° to 25°C . In this temperature range the crest time is known to be about 0.3σ . The corresponding durations of the action potential up to N_2 , in the great majority of the experiments, fell between $6-8\sigma$. The data justify the semi-quantitative statement that under the most favorable conditions the spike potential is evident for at least twenty to twenty-five times the duration of its rising phase.

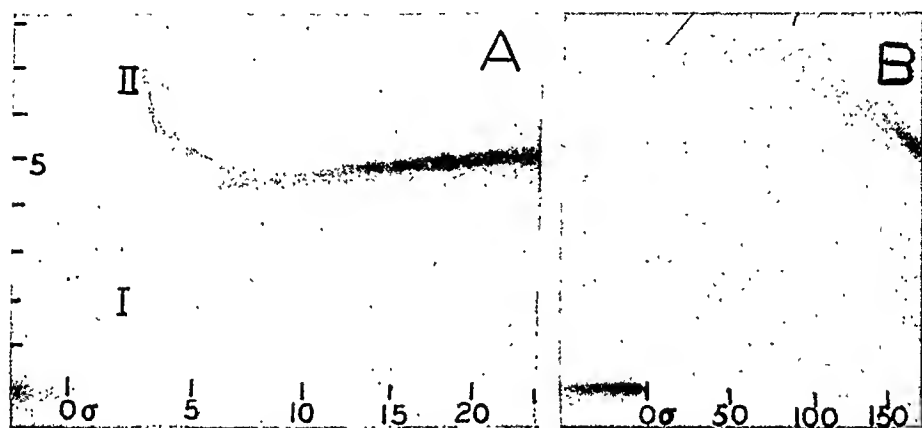


Fig. 4. A, change in form of the action potential as the lead shifts from diphasic toward monophasic. I, nerve treated with veratrin; II, same nerve immediately after stimulation. Ordinate, per cent of crest height. The pre-veratrinization form of the after-potential of this nerve is shown in figure 3, 3. B, like A but record continued over a longer period to show the crest of the after potential. The spike height does not correspond to that in A.

The part of the spike with the steep slope, that is, up to the bend on the falling phase, which is the part measured in low amplification records, is therefore only 11 to 15 per cent of the total detectable duration.

The magnitude of the end of the spike can only be approximated as lying between two theoretical limits. The upper limit is the value of the total potential in a cool fresh nerve which contains in addition to the spike a small undetermined amount of after-potential. The lower limit is obtained in an experiment of the following type. When a nerve is treated with veratrin, the form of the spike is unchanged, in contrast to the great increase in magnitude and duration of the after-potential (9). When such a nerve is subjected to a period of rapid stimulation, the after-potential may show a nearly stationary value in the portion under the end

Concomitant change of the spike and after-potential caused difficulty. When the nerve was warmed above laboratory temperature, the changes in the after-potential interfered less, and N_2 was easier to locate. On one nerve observed at 26° and 29.5°C. both the crest-time and the time to N_2 were 1.4 times as long at the lower as at the higher temperature; in another nerve, between the temperatures of 24.2° and 31°C. the coefficient was 1.5 for the spike and 1.6 for N_2 . N_2 was obviously behaving as one would expect it to behave as part of the spike.

The location of the end of the spike is possible in nerves in which a change occurs in the degree of diphasicity of the lead. When the lead is monophasic a greater addition of spike potential to the after-potential occurs than when the lead is diphasic, so that as a change is made from one condition to the other the combined potential should rise and fall, pivoting about the point N_2 which is postulated to mark the end of the spike. That this is the case is shown in figure 4A. The cause of the shift from diphasicity to monophasicity in this instance was a period of tetanic stimulation. The nerve had been treated with veratrin, and on several occasions we have noted that such nerves, though recording a second phase when rested, become nearly monophasic after stimulation. The stimulation must so alter the nerve at its killed end as to cause a suppression of the response in that region and thereby remove the second phase. In the spontaneous increase in the size of the second phase that occurs in the period following a fresh inactivation of the end of a nerve by heat, the same pivoting of the end of the spike has been noted.

Another bit of evidence as to the origin of N_2 is derived from the behavior of the T wave as the after-potential changes in shape. The T wave, that is the whole area of the spike between the diphasic artifact and the end, should move as a unit as the after-potential increases. Our records contain numerous cases of such movement. None of the combinations of records which show it is reproduced, but their nature may be illustrated from the figures. The fresh nerve shows a trace of the T crest and the N_2 trough, as in figure 3, 4; later when the after-potential is larger nearly the whole T wave becomes exposed, as in figure 3, 1, with the crest and trough in the same positions which they held in the first record.

Duration and magnitude of the end of the spike. Two questions are of interest with respect to the end of the spike: how long does it last and what is its potential. As the spike ends asymptotically no definite value can be given to its duration,—one can only say that it lasts at least as long as it can be detected as such. The best figures for the apparent end are obtained when the spike joins an approximately horizontal potential of such size and duration that there is no doubt that it is an after-potential.

Experimental examination of the origin of N_2 . Using a conventional monophasic lead (nerve killed by heat at the distal electrode) a number of procedures were tried to test the hypothesis that the spike lasts at least up to the position of N_2 . It was first necessary to show that N_2 is not an artifact. That it was not connected with the shock artifact was shown in a number of ways. The simplest and most direct way was to reverse the shock; this was done repeatedly in different experiments without making any significant change in the picture. The notch appeared as well when conduction was long as when it was short; and when records were made with the stimulus near the lead a comparison of them with the shock artifact recorded after subsequent narcosis, revealed that the notch could not be related to the artifact.

Another source of artifacts is the second lead, but if the notch were due to the second lead it should mirror some event at the first lead occurring earlier by the period of interpolar conduction. No event fulfilling this condition is observable; furthermore, when the nerve is so completely monophasic that no distortion at all can be detected in the spike the notch is still visible. The additional possibility that N_2 is an artifact produced by irregularities in the nerve between the leads is readily ruled out.

Theoretically, to prove that N_2 is produced by the ending of the spike it is necessary to show that it has the qualities which would be predicted from the known behavior of the spike. In going over the possible tests it was found that some of the more obvious ones were not applicable. For instance, N_2 should come later when the conducting distance is long, but its position is too ill-defined for this small difference to be measurable. The change of the form with temperature proved better, but here the si-

Fig. 2. Action-potential curves which would be produced by combining the spike shown in figure 1 with after-potentials of various arbitrary forms. Abscissae, time in sigmas; ordinates, potential in percentage of spike height. Interrupted line, after-potential; solid line, spike alone or summed with after-potential. A, B, C, partially diphasic; D, E, F, monophasic.

Fig. 3. Action-potentials recorded under various conditions in such a way as to show the transition from the spike to the after-potential. The potential is calibrated in percentage of spike height (ordinate). The time is marked in 5 sigma intervals. Reproduction $\times 0.7$. The spike starts at the break in the line but only its end is visible. Records 1-5, partially diphasic; 6 and 7 monophasic. 1 and 2, 11/23/31, 26°C., 4 mm. conduction; 2, normal nerve, response 18 per cent of maximum; 1, same nerve after application of veratrin and stimulation, response 11 per cent of maximum. 3, 12/1/31, 22°C., 3 mm. conduction, normal nerve, response 60 per cent of maximum. 4, 11/17/31, 25.5°C., normal nerve, response about 20 per cent of maximum. 5, 12/7/31, 30°C., 10 mm. conduction, normal nerve, response 40 per cent of maximum. 6, 1/6/32, 22.2°C., normal nerve, lead from cathode, nearly maximum. 7, 1/7/32, 25°C., nerve treated with veratrin and stimulated. The nerve had been made monophasic with potassium and its monophasicity proven in earlier observations.

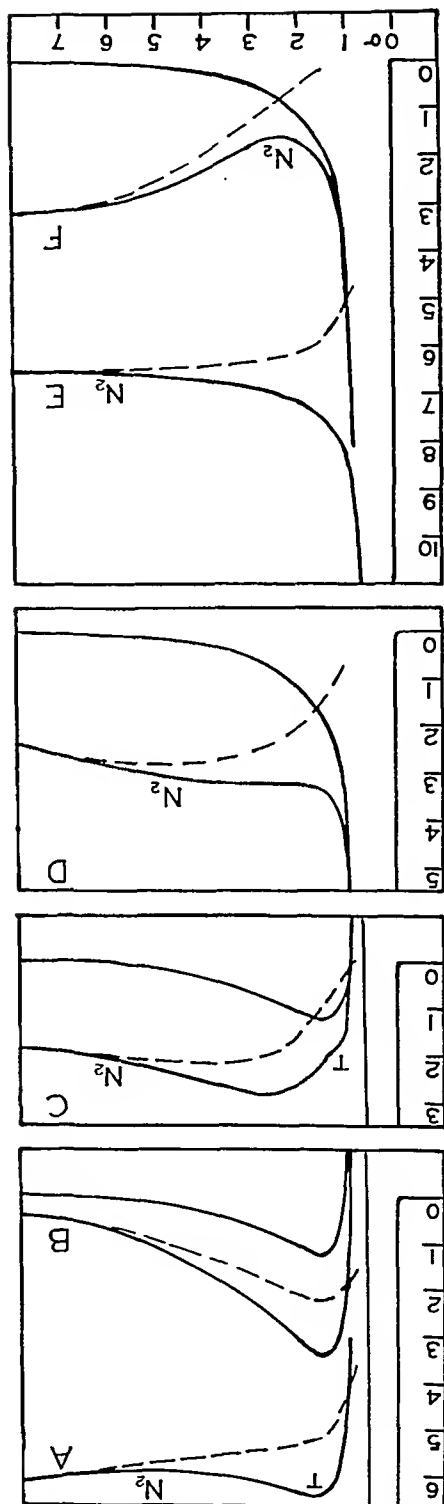


Fig. 2

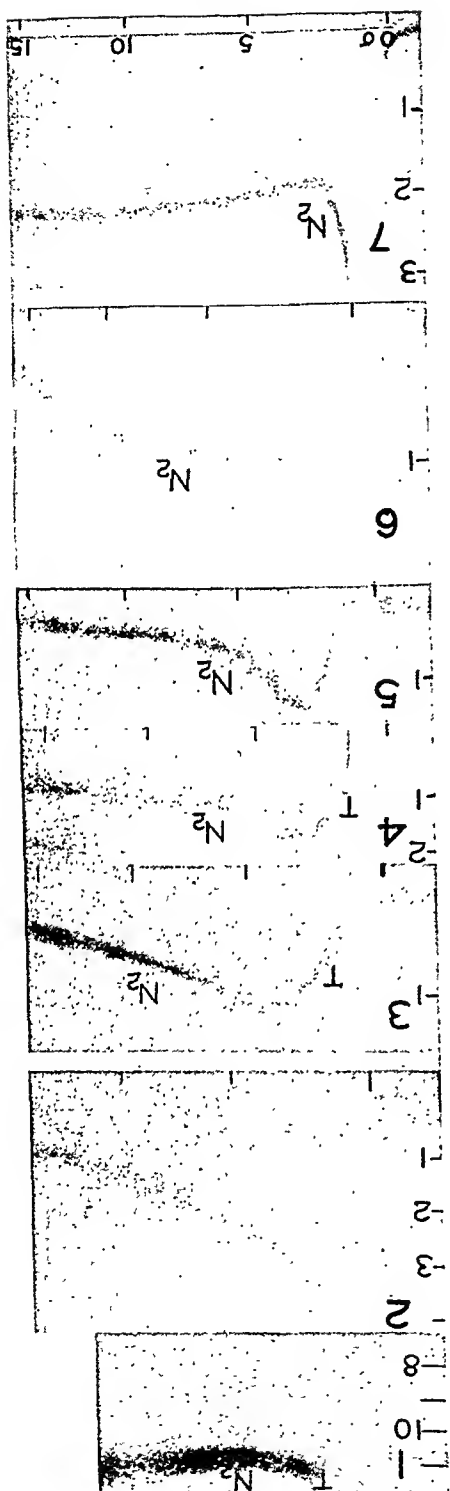


Fig. 3

spike form constant except for height; the potentials recorded are thus the sum of a constant¹ and a variable. In order to facilitate a clearer understanding of the various ways that the spike and after-potential may fuse, some of the possibilities are drawn in figure 2. For the portion belonging to the spike the monophasic ending shown in figure 1 is chosen as sufficiently representative; in each instance it or its diphasic derivative is added to an after-potential of arbitrarily assumed form to give the combination shown. Beside the synthetic curves is given a series of action potential records (fig. 3) made from sciatic nerves of *rana pipiens* under the conditions stated in the legend.

In figure 2B the after potential is assumed to be small and to have a maximum very close to the spike. With it the T wave of the spike fuses in such a way as to be undifferentiable; neither the T wave nor the end of the spike can be distinguished, the former because of the similarity of its shape to that of the after-potential at the same point, and the latter because of the close approximation of its slope to that of the after-potential. If we now imagine that the after-potential changes to the form shown in figure 2A, then the T wave emerges and the spike-end becomes visible because in the summed curve a negative slope is maintained by the spike until the latter becomes so attenuated that the slightly positive slope of the after-potential becomes dominant. The curve thus runs through a minimum (N_2). The first and second curves of figure 3 which were taken from the same nerve before (2) and after (1) treatment with veratrin have forms similar to the derived curves B and A.

In figure 2C the after-potential is assumed to rise more slowly at its start; therefore there is a suggestion of the T wave in the summation, and the slopes of the two components are just different enough to produce a change in direction of the line where the after-potential is no longer overlaid by the spike. The third, fourth and fifth curves of figure 3 resemble this type of combination.

Figure 2D may be compared with curve 6 in figure 3. The end of the spike—in this case monophasic—is not visible; nevertheless the spike may be traced to a point somewhat short of its end because of the point of inflection it causes in the curve of addition with the after-potential. It is logical to assume that the spike lasts at least until the curve begins to show negative concavity.

When the after-potential rises more slowly still (fig. 2F) the notch becomes very definite but occurs considerably before the end of the spike. This is the form of curve 7 of figure 3.

The time of termination of the spike can best be evaluated when the spike ends on a flat after-potential (fig. 2E).

¹ In practice the amount of diphasicity actually occurring in a monophasic lead may vary with the momentary condition of the nerve. This changes the recorded form of the spike and adds considerably to the difficulty of interpretation of records.

In actual experiments the curves obtained may vary considerably from the one shown in the figure because of other conditions. The relative magnitude to which the artifact is manifest will be altered by the interpolar distance and by temporal dispersion of the second phase. The theoretical curve is derived for a rather long interpolar distance; distances longer or shorter than this would make the second phase more or less apparent. Temporal dispersion, which is inevitable in the second phase even if all the impulses start from the first lead simultaneously, will make the second phase more apparent by causing it to be recorded under a lower part of

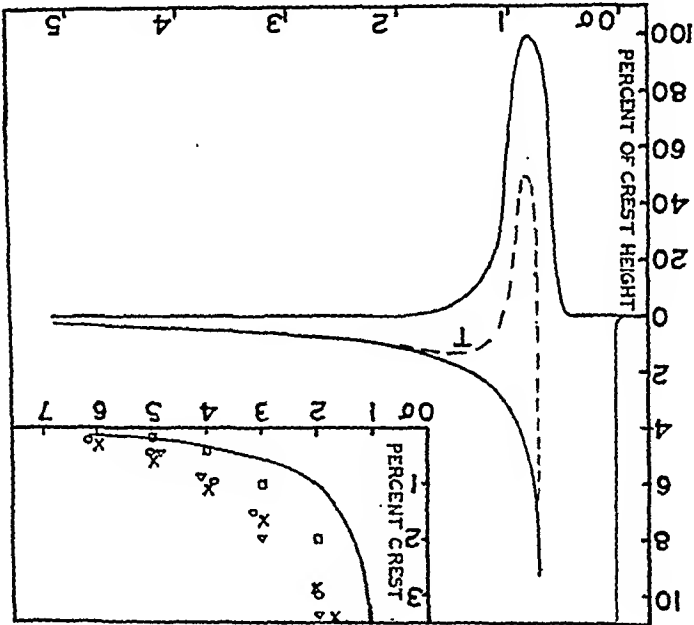


Fig. 1. Partially diphasic response reconstructed from a monophasic spike determined for the purpose, 12/5/31. Inset: Points—end of spike as measured from the level of flat veratrin after-potentials. Solid line—monophasic spike from main figure drawn in for comparison.

the potential at the first lead. In nearly all the experimental variations there is some negative potential at the end due to a residual dominance of the first phase. This results in the appearance of two crests in the axon spike potential. To distinguish the second crest from waves produced by the fiber constitution of the nerve, it has been designated the T wave for purposes of reference, in analogy with the T wave of the electrocardiogram, as the latter is interpreted on the interference theory.

Comparison of theoretical reconstructions and recorded action potentials. At constant temperature, the various experimental conditions to which a nerve may be subjected change the after-potential but leave the axon

but a theoretical reconstruction soon shows that it is a natural consequence of the form of the spike.

The monophasic spike. For reconstruction purposes it is necessary to employ a form of the spike in which there is little distortion at the end on account of diphasicity. Previous methods of approximating this have been to locate the diphasic artifact directly under the first phase as the result of a very short interpolar distance (6), or to place the stimulus near the second lead so that the front rather than the tail of the wave is distorted (Bishop, 1927). Recently another method has become available as the result of a chance observation. It was noticed that nerves, which had been heat coagulated at the end for a monophasic lead, lost their residual diphasicity when the whole nerve was painted with Ringer's solution rich in potassium. This suggested that a nerve treated with potassium at one lead would be more monophasic than one prepared by crushing or heating, and such proved to be the case. The only difficulty is the danger of spread of the potassium along the nerve to the active lead, an event easily leading to erroneous conclusions in after-potential experiments since potassium decreases the after-potential more than it does the spike (8).

Another desirable quality in the spike form used for reconstruction purposes is freedom from after-potential, but no means of obtaining this is available: the schemes for reducing the after-potential are only differential with respect to the spike. The after-potential can be held to a small value, however, if a nerve be used fresh and kept at a cool temperature. The spike used in the following reconstruction and reproduced in figure 1 (solid line) was obtained at 14.2° from fresh frog nerve made monophasic with potassium. The experiment was performed under conditions eliminating temporal dispersion and the final form was synthesized from records made at two amplifications, one for the form of the early high portion and the other for the decremental tail. The observed crest time was 0.82σ ; but inasmuch as at the ordinary experimental temperature 0.3σ is the usual figure the spike was calculated to this value. This procedure is justifiable because the relative form is not altered by temperature changes (6).

Derivation of the diphasic spike from the monophasic. The graphic derivation of the theoretical form of a diphasic response is shown in figure 1. The lower part of the monophasic spike described in the preceding paragraph is plotted above the zero line. On the assumption that the second phase is 10 per cent of the first and starts 0.5σ after it, the same spike calculated to the designated relative size is plotted in the appropriate position and in the reverse direction below the line. The latter curve gives the complete form of the spike as obtained in the experiment. The algebraic sum of the two curves—shown as an interrupted line—gives the form of the corresponding diphasic spike.

STUDIES ON ALBUMINURIA FOLLOWING EXERCISE

I. ITS INCIDENCE IN WOMEN AND ITS RELATIONSHIP TO THE NEGATIVE PHASE IN PULSE PRESSURE

FRANCES A. HELLEBRANDT

From the Department of Physiology, University of Wisconsin, Madison, Wisconsin

Received for publication March 31, 1932

White clouds in the urine had been noted for centuries before Richard Bright interpreted the significance of this finding and published his epoch-making description of essential nephritis. Fifty years passed before clinical men began to examine the urine of the apparently healthy and it became known that albuminuria might occur unassociated with the signs and symptoms of nephritis. Protein in the urine was found to be a frequent concomitant of pubescence and an inferior constitutional build. Pavy (1886) early pointed out the peculiar relationship of the upright position to this anomaly of adolescence, and Stirling (1887) suggested the name "postural albuminuria." Subjects who suffer from this albuminuria frequently have an exaggerated lumbar lordosis, and Rieser and Rieser (1922) presented the hypothesis that this postural defect mechanically interferes with renal circulation, producing a stasis which makes the kidney permeable to albumin. Erlanger and Hooker (1904) made an extensive study of a case of orthostatic albuminuria and found that the diminution in pulse pressure with the assumption of the upright posture was the only factor consistently related to the output of albumin. Dunhill and Patterson had already observed that albuminuria might follow upon exercise, and Lowsley (1911), studying the cardiovascular responses to various types of physical activity, noted that post-exercise albuminuria was associated with the occurrence of a subnormal drop in pulse pressure.

The general opinion among clinicians and laboratory investigators has been that these types of albuminuria are benign, being based upon no permanent underlying renal disease; such kidneys are generally considered functionally as competent as the impermeable kidney. To call such intermittent albuminuria functional implies it to be a physiological response. The evidence of the benign nature of these albuminurias is not conclusive merely because they are not the obvious precursors of nephritis. They should not be dismissed before their cause has been more perfectly elucidated. Few studies have concerned themselves with the incidence of post-

exercise albuminuria in the female. The purpose of this study is to determine the frequency with which it occurs after experimental exercise in women, and to study its relationship to the post-exercise fall in pulse pressure.

Incidence of post-exercise albuminuria. From 47 individuals, analyses were made of the urine collected immediately following experimental bouts of physical exertion of various types, such as riding the bicycle ergometer, running around an indoor track at top speed, and working in a rowing machine. Of the subjects studied, 14.8 per cent showed albumin in a sample of urine voided before the onset of exercise. This pre-exercise albumin did not always increase in quantity after physical exertion. Once it remained unchanged and three times reduced in amount. Of those who had no albumin in the urine before exercise, physical work produced an albuminuria in 57.5 per cent and its severity was inversely proportional to its frequency of occurrence.

Relationship of post-exercise albuminuria to the negative phase. In this laboratory Henry and Dawson (Henry, 1928) had investigated the negative phase following work on a bicycle ergometer and had confirmed the observations of Lowsley (1911) who found that there is a marked elevation of pulse pressure during exercise, followed by a diminution to a level below the pre-exercise normal after the cessation of muscular activity. This period of subnormal pulse pressure has been termed the negative phase. The bicycle ergometer permits a bout of exercise that can be readily adjusted in severity to throw the subject into a post-exercise condition with a cardiovascular reaction characterized by a diminution in the amplitude of the pulse pressure.

The standard dosage of work was a half-hour ride on a bicycle ergometer with a Prony brake, the tension on the brake band being kept constant at three pounds. The subjects adjusted the severity of the work to their own fitness by riding steadily at individual maximal speed. Observations were made upon twenty-three subjects. Those upon whom the primary work of the study was conducted were young women physical education students, familiar with physiological experimental technique and coöperative to an unusual degree. As a whole the group might be characterized as one accustomed beyond the ordinary to strenuous physical exertion.

The primary object of these experiments was to determine whether or not post-exercise albuminuria is produced by a diminution in pulse pressure to a level below normal. It was necessary to obtain three urine samples: first, a urine sample before the onset of exercise as a standard for comparison; second, a urine sample immediately at the cessation of exercise to determine whether or not the kidney becomes permeable to protein during the period of active waste production and the phase of hypertension with high pulse pressure; third, a sample of urine produced by the kidney during the

post-exercise period of negative phase when the pulse pressure sinks sub-normally.

There was frequent difficulty in the collection of urine samples, both before the bicycle ride and particularly, immediately after. The first of these was a psychic inability to void, which disappeared spontaneously after the subject became familiar with the experimental procedure. It was difficult to determine whether the inability to collect urine samples after exercise was also psychic or due to an exercise anuria. Following the suggestion of the procedure routinely adopted when the phenolsulphonaphthalein test of kidney function (Todd, 1925) is given, the subject drank 200 cc. of water after she had collected her first sample of urine and approximately one-half hour before the onset of the ride. This sufficiently promoted kidney secretion and henceforth in the majority of cases samples immediately after exercise were successfully collected.

The details of the standard technique follow: The subject came to the laboratory and changed into riding clothes. Urine sample 1 was collected, the bladder being completely emptied. Two hundred cubic centimeters of water were taken by mouth. After cardiovascular stabilization had occurred, systolic and diastolic pressures were determined, and the pre-exercise pulse pressure was recorded. The subject began to ride the bicycle ergometer twenty to thirty minutes after the intake of the water. When the subject became heated, an electric fan was turned on and subsequently the laboratory windows were widely opened to insure a sufficiently low room temperature and a free circulation of air. The subject rode for one-half hour. Immediately at the cessation of the ride, urine sample 2 was collected, the bladder being again completely emptied. The subject drank a second 200 cc. of water and was well covered to prevent chilling. Blood pressure readings were commenced and the time of onset of the negative phase was recorded. Readings were usually continued until the pulse pressure returned to normal, the subject remaining quietly seated throughout this procedure. When the negative phase was protracted, urine sample 3 was collected before the return of the pulse pressure to normal, since the period was sufficiently long to permit the kidney to secrete an adequate sample of urine.

The urine samples were tested for albumin by two methods: heat and 3 per cent acetic acid, and by salicylsulphonic acid. McNabb and Field (1924) had found considerable difference in the sensitivity of the various urinary albumin tests in common use by clinical laboratory diagnosticians. The heat and acetic acid test was, in their hands, the most satisfactory. They found the salicylsulphonic acid test next to heat and acetic acid in its delicacy. The technique suggested by McLean (1924) was used. Six drops of a saturated aqueous solution of salicylsulphonic acid were layered upon a half inch of urine in a test tube. Without heating, large amounts

of albumin immediately formed a dense white precipitate, while small amounts produced a definite milky or an easily detectable opalescence.

There were twenty-five experiments with records of both blood pressure and urinalysis findings. Of these, five were eliminated from the pulse pressure study because the subjects showed albumin in urine sample 1 collected before the onset of exercise. The results of five more were cast out because the subjects were unable to obtain urine sample 2. Several of these were contributed by one subject, an experienced laboratory worker who was never able to void immediately after the cessation of the exercise. One was eliminated because albuminuria appeared immediately after the ride. This phenomenon was subsequently studied and is discussed in the second paper.

The results may be summarized as follows: *a.* Eight experiments demonstrated a fall in pulse pressure unassociated with albuminuria; the average pulse pressure drop was 11.2 mm. of mercury below normal, and the average duration of the negative phase was 51 minutes. *b.* Six presented a fall in pulse pressure with a concomitant albuminous urine, confined solely to urine sample 3, secreted during the negative phase; the average pulse pressure drop was 21.3 mm. of mercury below normal and the average duration of the negative phase was 72 minutes. This is a diminution 90.1 per cent greater than the depth of the negative phase and 41.8 per cent longer than that occurring in the group whose kidneys did not become permeable to protein.

To confirm the evidence of an etiological relationship between post-exercise albuminuria and the negative phase, a subject was trained to void at short intervals of time without change in position and cardiovascular disturbance. To aid in the secretion of adequate samples, 50 cc. of water were administered at constant intervals, at the time of the collection of urine samples. All urine samples were centrifuged and tested quantitatively for albumin, using the sulphosalicylic acid turbidimetric method of Kingsbury, Clark, Williams and Post (1926).

The subject was well stabilized in the sitting posture. The bladder was then completely emptied and with the administration of the first 50 cc. of water, observations were commenced, blood pressure readings being made at sixty second intervals and urine samples collected every twenty minutes. Pre-exercise blood pressure was observed during the first twenty minute experimental period. After voiding the pre-exercise urine sample, the subject rode the bicycle ergometer for twenty minutes, doing a moderately severe piece of work at a pedalling rate which averaged 80 revolutions per minute. Immediately at the cessation of the ride, the exercise urine sample was voided and blood pressure readings were resumed. Systolic and diastolic pressure fell steadily and there was a very moderate reduction in pulse pressure during the first twenty-two minutes after the exercise. The

diastolic pressure then began to drop precipitously and a less acute fall in systolic pressure occurred. This terminated in a syncopal attack. Blood pressure readings were resumed with the subject in the recumbent position. The subject slept during the succeeding hour, being aroused periodically for the collection of urine samples. The pulse pressure fell gradually and then slowly approached the pre-exercise level. After the return trend had been established, the subject was again placed in the sitting posture. A secondary pulse pressure drop occurred with the assumption of the vertical position. Observations ceased three hours after the termination of the ergometer ride.

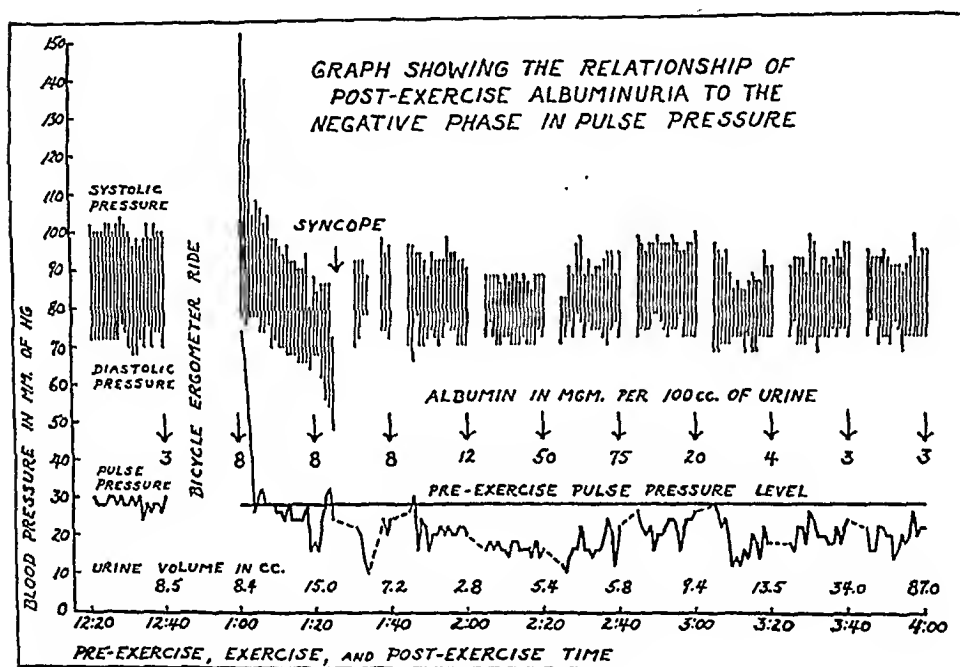


Fig. 1

The accompanying graph shows the pre- and post-exercise cardiovascular reactions, the amount of urine secreted and the quantity of albumin produced during each twenty minute period. The greatest amount of albumin appears during the period of most marked oliguria and the maximal reduction in the amplitude of the pulse pressure.

These findings, which indicate a relationship between the negative phase and albuminuria, may possibly be explained by suggestions that come from recent work on the physiology of the kidney. In view of his observations on spontaneous interruptions occurring in the circulation of individual glomeruli (Richards and Schmidt, 1924), Richards (1925) believes that when a constriction stimulus reaches the arteriole and produces its partial clo-

sure, the wall of the vessel suffers from an oxygen deficiency which depresses it directly. The cells of the tubules surrounding the affected arteriole are subsequently also subjected to anoxemic conditions. This oxygen deficiency institutes the production of substances which have a vaso-dilator effect. The period of intermittence in any single glomerulus is, under normal conditions, so short that there is no anoxemic damage to the endothelium of the glomerulus and no albumin appears in the urine. Richards suggests that albumin might thus result under conditions not far removed from the physiological, when for any reason the duration of the intermittent cessation of glomerular flow increases. Many years ago, Herrmann (quoted by Fischer, 1912) had shown that local circulatory disturbances of the kidney produce an albuminuria, and the later experiments of Araki and of Zillessen (Fischer, 1912) demonstrated that a diminution of the oxygen supply to any parenchymatous organ, through interference with its normal circulation, is followed by a localized accumulation of acids. Wearn and Richards (1924) introduced a quartz needle into Bowman's capsule and removed urine secreted by the glomerulus. They observed that when the blood flow through the glomerular capillaries was rapid, there was no albumin in the urine secreted but that when the circulation was sluggish and the capillaries were dilated, the urine became albuminous.

During exercise there is a marked elevation of the pulse pressure, an increase in the circulation rate, and a shunting of blood to the active muscles and the skin. Muscular exercise elevates the level of the metabolism and concomitantly the production of heat. If the exercise is severe enough the production of heat may exceed the heat loss and the temperature may rise. When the bicycle ergometer was ridden with a heavy load at a high speed for as short a time as two minutes, the body temperature elevated 0.1° to 0.4°F. during nine out of ten consecutive rides. There is normally a balance between the functional activity of the kidneys and the skin. The kidneys become relatively inactive when large amounts of water are being lost through the skin, one of the most efficacious of the channels of heat loss. Some of the subjects remained pale throughout the course of the half hour ride and experienced little sweating. Others became flushed, with an irregularly distributed dull red blotching, most commonly confined to the face, arms and thighs. The subjects in whom visible perspiration was profuse exhibited marked dilatation of the peripheral vessels in the skin, and it was observed that the incidence of post-exercise albuminuria was greatest in the cases with such peripheral change. The shunting of large volumes of blood to the skin in addition to the active muscles in the extremities reduces the flow of blood through the visceral area. Those in whom the peripheral dilatation is excessive have shunted more blood away from the kidneys and under these circumstances, the intermittence of glomerular circulation may persist beyond the limits of normal and produce an asphyxial damage of the kidney.

After exercise ceases, temperature equilibrium is reestablished at a lower level and cardiovascular recovery takes place. The systolic pressure falls rapidly to a point below normal. The diastolic pressure is slower to fall and the pulse pressure drops. There is frequently a secondary rise in diastolic pressure coincident with the maximum pulse pressure fall. The circulation in the kidneys is sluggish until sufficient time elapses for the reestablishment of a proper balance between the systolic and diastolic pressures. Acids accumulate in the cells of the kidney, and when the glomeruli open, blood proteins escape.

Two causes therefore may combine to permit a leakage of albumin through the glomerular endothelium; first, asphyxiation during the exercise; second, sluggish circulation after the exercise. In those subjects in whom there occurs excessive peripheral dilatation, and in whom a deep and protracted fall in pulse pressure follows upon the cessation of exercise, we find the highest incidence of post-exercise albuminuria.

SUMMARY

1. The incidence of albuminuria increased from 14.8 per cent before exercise to 57.5 per cent after exercise.

2. The pulse pressure fell below normal in all cases after a half-hour bicycle ergometer ride.

3. The pulse pressure fall in the cases with post-exercise albuminuria was 90.1 per cent greater in depth and was maintained 41.8 per cent longer than the pulse pressure fall in the cases without albumin following physical exertion.

4. The greatest amount of albumin was found to occur during the period of oliguria and the maximal reduction in the amplitude of the pulse pressure.

5. The experimental results may be explained by the hypothesis that strenuous exercise shunts the blood to the working muscles and the skin, affecting the circulation of the kidney in such a way as to cause asphyxiation of the renal cells beyond that compatible with normal function. The deep and protracted post-exercise low pulse pressure adds to the anoxemia already present and induces an abnormal accumulation of acids in the renal tissue which alters its permeability to the blood proteins and albumin appears in the urine.

BIBLIOGRAPHY

- ERLANGER, J. AND D. R. HOOKER. 1904. Johns Hopkins Hosp. Repts., xii, 145.
FISCHER, M. H. 1912. Nephritis. John Wiley & Sons, New York.
HENRY, F. A. 1928. Unpublished thesis, University of Wisconsin.
LOWSLEY, O. S. 1911. This Journal, xxvii, 446.
MCLEAN, H. 1924. Modern methods in the diagnosis and treatment of renal disease. Constable & Co., London.
McNABB, P. E. AND C. W. FIELD. 1924. The Military Surgeon, iv, 73.

PAVY, F. W. 1886. *Lancet*, i, 437.

RICHARDS, A. M. 1925. *Journ. Urology*, xiii, 283.

RICHARDS, A. M. AND C. I. SCHMIDT. 1924. *This Journal*, lxxi, 178.

RIESER, W. AND S. L. RIESER. 1922. *Journ. Amer. Med. Assoc.*, lxxviii, 644.

STIRLING, A. W. 1887. *Lancet*, ii, 1157.

TODD, J. C. 1925. *Clinical diagnosis by laboratory methods*. W. B. Saunders Co., Philadelphia.

WEARN, J. T. AND A. N. RICHARDS. 1924. *This Journal*, lxxi, 219.

STUDIES ON ALBUMINURIA FOLLOWING EXERCISE

II. ITS RELATIONSHIP TO THE SPEED OF DOING WORK

FRANCES A. HELLEBRANDT, ELIZABETH BROGDON AND L. E. A. KELSO

From the Departments of Physiology and Electrical Engineering, University of Wisconsin, Madison, Wisconsin

Received for publication March 31, 1932

In the course of the studies concerning post-exercise albuminuria in its relationship to the negative phase in pulse pressure, it was observed that when the exercise was severe, exhausting and carried on with intermittent bursts of speed, albumin appeared in the urine immediately after exercise and there was an apparent reduction in its amount during the subsequent period of low pulse pressure. The albuminuria following moderate, prolonged and steady exercise had been confined solely to the period of low pulse pressure. The purpose of this study is to ascertain whether there is any relationship between albuminuria and the speed of doing work.

The augmentation of the metabolic activities of the tissues, the physico-chemical changes in the blood and the magnitude of the cardiovascular responses to exercise are all related to the speed, duration and severity of the muscular exertion. They may be used as indices of the cost of the effect achieved for they bear a relationship to the effort put forth. Post-exercise albuminuria may be a physiological response or it may be the concomitant of exercise too severe to be tolerated without pathological change. The presence of albumin in the urine or the failure of post-exercise albuminuria to occur cannot be evaluated without quantitative data concerning the work done.

In a physical sense time is not a factor which enters into the estimation of work, but physiologically it is as important to know with accuracy the rate at which work is being done as to know the work accomplished. In practice the Prony brake ergometer was found unsatisfactory for accurate quantitative estimations of the rate of working. The mechanical power input of the Prony brake is transformed by friction into heat and dissipated. Its coefficient of friction varied so markedly during any given run that the instrument could not be used without frequent load adjustment and the constant assistance of an operator. A new bicycle ergometer was therefore developed on the electrodynamic brake principle.

In the electrodynamic brake bicycle ergometer the mechanical power of

pedalling is transformed into electrical power which in turn is converted into heat and dissipated into the surrounding medium. The fields of a small dynamo are excited with a constant current from a storage battery, generating a flux which is cut by the conductors on an armature rotated by the movements of the bicycle pedals. This cutting of the flux generates a voltage which bears a fixed relationship to the rate of pedalling.

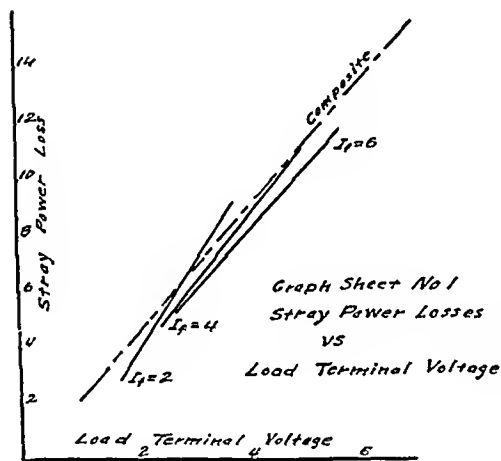
A standard woman's bicycle frame was firmly fixed by metal braces to a stout base-board. A twelve volt Dodge motor starter was mounted under the steering fork and arranged to be driven directly by the pedals through racing sprockets having 80 and 14 teeth, giving a gear ratio of 1 to 5.71. The load is the electric generator. A cast-iron fly-wheel was attached to the armature. The generator was connected for separate excitation of the shunt field, the series field having been disconnected. The exciting current was furnished by storage batteries and controlled by a sliding-contact rheostat placed within easy reach of the subject. The field current was indicated on a distant reading ammeter mounted on an upright and placed directly in front of the subject. The generator was loaded on a fixed resistance of 0.151 ohm, the value of which is independent of temperature. A distant reading voltmeter was connected across the load resistance and mounted on the upright above the ammeter. The voltmeter reading for any field current is controlled by the speed of pedalling. The magnitude of the load and the rate of pedalling are both indicated on the voltmeter. The ammeter gives the relation between the magnitude of the load and the rate of pedalling.

The mechanical input of the ergometer is used up in overcoming the rotative losses and in supplying the power generated in the armature. The rotative losses are made up of windage, the mechanical friction of the bearings and the chain, and the eddy current and hysteresis losses. They are determined by the electrical input method. The generator is run as a motor at various speeds and field currents and at no load. The stray power losses are practically independent of the load and vary only slightly with the different field currents and speeds. Graph 1 shows the stray power losses plotted against the generated voltage. The curves for the different field currents nearly coincide. Since they vary almost as the generated voltage, the rotative losses are considered as proportional to this voltage. The error incurred in estimating them as such is negligible.

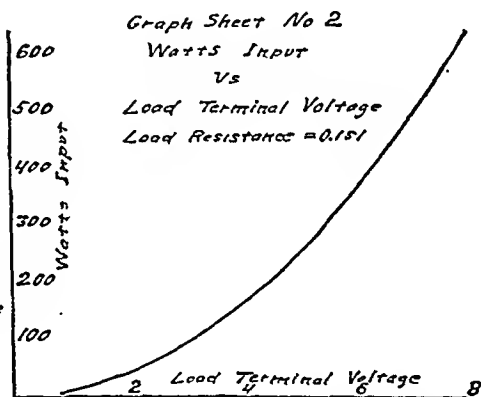
The generated power is the product of the armature current and the generated voltage. It is the delivered power plus the armature copper loss. The copper loss is computed from the armature resistance and the current. A simple equation has been developed for the calculation of the generated power in terms of the machine constants and the terminal voltage; to this the rotative losses are added and the total input is obtained. This is a function of the load terminal voltage. Since the load resistance

is constant, the armature current is proportional to the voltmeter reading; since for any field current, the rate of pedalling is also practically proportional to the voltmeter reading, the speed and load will be constant for any fixed field current and voltmeter reading. Graph 2 is a calibration curve for the ergometer. The load in watts for any voltmeter reading is not affected by the field current and therefore the curve is independent of the ammeter reading. It shows the total watts input as a function of the voltmeter reading. For the same power input a change in excitation will modify the rate of pedalling. If the pedalling is to be fast, the field current is made low. A high field current requires a low rate of pedalling for the same power input.

In actual operation the subject sets the field current at an assigned value and rides at such a rate that the voltage is held constant at another assigned



Graph 1

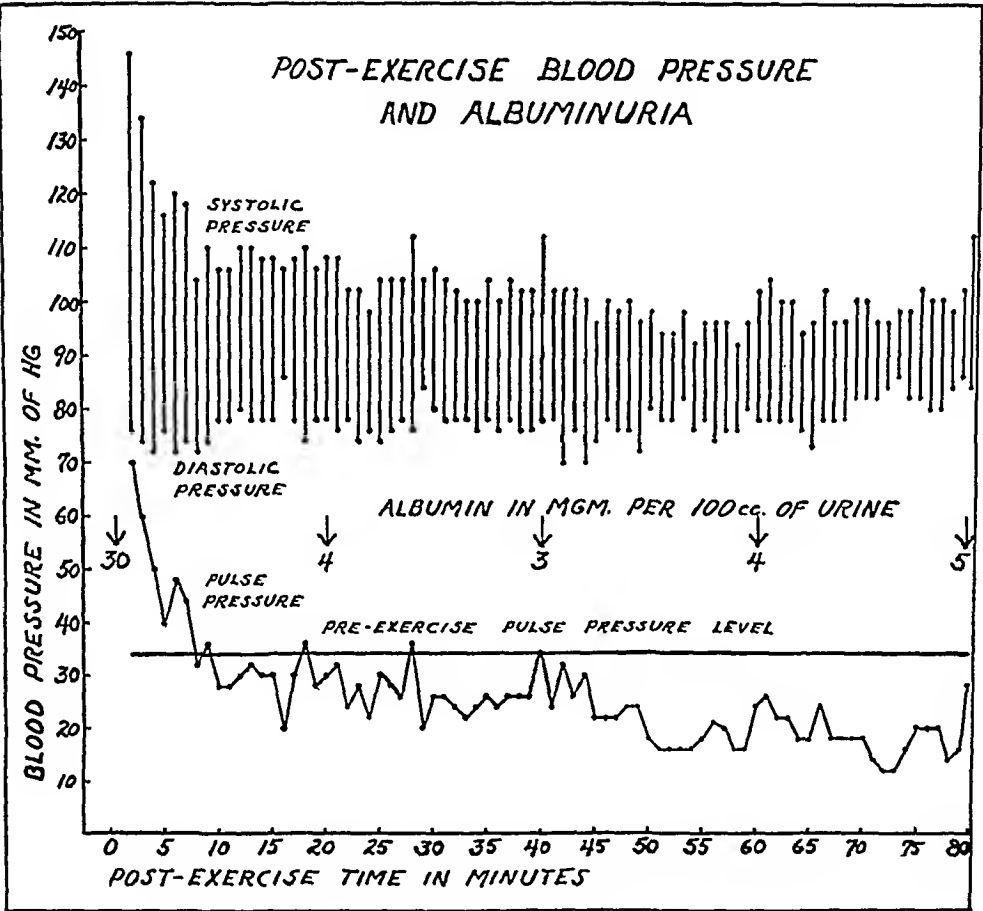


Graph 2

value. Knowing the load terminal voltage, the watts input may be read directly off the graph which accompanies the instrument, and if desired, converted into horse power or kilogram-meters per unit time. It is not sufficient to keep the average speed constant; the instantaneous speed must be unchanging. If two runs are made at the same *average* speed but with different *instantaneous* speed, the average powers may not be alike. The voltage must be kept constant. In practice it varied slightly but because of the fly-wheel effect on the armature shaft, the inexperienced subject was observed to closely approximate the steady pedalling of the experienced rider. The average speed of pedalling may be checked by recording the total revolutions of the armature shaft from ride to ride. The apparatus permits a bout of exercise to be repeated with very great accuracy any number of times, and the power input may be easily deter-

mined. It adapts itself especially well to the repetition of equivalent amounts of work at varying speeds.

The time consumed in doing work modifies greatly its cost and it is common knowledge that it is more expensive in terms of energy input to go fast than slow. As noted, it had been observed that long and violent rides carried on with intermittent bursts of speed were associated with an immediate post-exercise albuminuria. Graph 3 shows the cardiovascular



Graph 3. Showing an immediate post-exercise albuminuria and the increase in albumin during the negative phase in pulse pressure.

response to such a bout of exercise. The subject was flushed, dyspneic, and exhausted at the termination of 25 minutes of pedalling. A urine sample voided immediately after the cessation of work contained 30 mgm. of albumin per 100 cc. The subject voided at 20 minute intervals. The first two samples collected showed a marked reduction in the amount of albumin present. The post-exercise cardiovascular response was typical, the pulse pressure diminishing in amplitude to a value below the pre-

exercise level. With the establishment of the most pronounced period of negative phase a slight but steady increase occurred in the milligrams of albumin present per 100 cc. of urine. The absolute amount of albumin continued to rise in the urine secreted during three successive 20 minute intervals following the cessation of blood pressure readings. There seem to be two albuminurias occurring, one during the exercise and the other during the post-exercise period of low pulse pressure.

With the use of the electrodynamic brake bicycle ergometer, various bouts of exercise were studied, to observe especially the influence of speed of working upon the incidence of albuminuria. It failed to occur after the types of exercise recorded in table 1. The exercises were all carried on at a slow or moderate pedalling rate. After the two long rides cardiovascular stabilization occurred rapidly and no lasting negative phase appeared. The short rides were very severe because the field currents were

TABLE 1

Showing the speed, duration and severity of various bouts of work which failed to produce albuminuria

TRIAL	SUBJECT	PEDALLING RATE	FIELD CURRENT	LOAD TERMINAL VOLTAGE	DURATION OF RIDE	RATE OF WORKING
		<i>r.p.m.</i>	<i>amperes</i>		<i>minutes</i>	<i>kgm.m/min.</i>
1	B. L	48	8.0	3.0	5	547.8
2	B. L	52	6.0	3.0	5	547.8
3	F. A. H.	61	3.4	2.4	60	365.3
4	B. L.	64	7.0	4.0	2	976.91
5	F. A. H.	68	6.0	4.0	5	976.91
6	E. L.	70	4.0	3.0	30	547.8
7	E. L.	75	5.0	4.0	5	976.91

relatively high. Post-exercise urine was collected approximately twenty minutes after these rides to allow sufficient time for the collection of an adequate sample. During this brief post-exercise period there were as yet no evidences of a deep negative phase, the reductions in the amplitude of the pulse pressure being irregular and evanescent.

In 1911 Lowsley had demonstrated on seven cases that a relationship exists between the post-exercise fall in pulse pressure and albuminuria following exercise, and had found that the quantity of protein appearing in the urine is greater the more extensive the period of low pulse pressure. He noted that short, rapid and exhaustive exercise tended to produce a marked and prolonged negative phase. With these considerations in mind, short rapid and severe bouts of exercise were instituted and the amount of albumin so induced was quantitatively determined.

The subjects of the experimentation were young women between the

ages of eighteen and thirty, either university students or faculty members. The group as a whole was above the average in neuro-muscular development, all having had professional training in physical education. They were familiar with physiological experimental technique and had all ridden the bicycle ergometer at some time prior to this research.

Immediately upon coming to the laboratory the subjects collected urine sample 1 and then sat on the bicycle until cardiovascular stabilization had occurred. The field current of the electrodynamic brake ergometer was set to give the desired load and the subject kept her speed of pedalling constant by maintaining the voltmeter needle on a given point. The revolutions per minute were estimated from the readings of a counter actuated by an eccentric on the armature shaft. Since not enough urine

TABLE 2

Showing the relationship of post-exercise albuminuria to the depth and duration of the negative phase within twenty minutes after a series of bicycle ergometer rides of the following speed, duration and severity

Field current—3.2 amperes. Load terminal voltage—3.5 volts. Pedalling rate—98 R.P.M. Duration of ride—5 minutes. Rate of working—762.36 kgm.m/min. Total work done—3,811.8 kgm. m.

	RIDE									
	1	2	3	4	5	6	7	8	9	10
Negative phase in pulse pressure during first 20 minutes post-exercise:										
Duration in minutes.....	0	0	0	1	3	5	6	7	9	10
Maximal depth in mm. Hg.....	0	0	0	2	4	10	17	8	7	8
Albumin in mgm./100 cc. of urine, 20 minutes after cessation of the exercise.....	48	25	47	55	49	35	30	10	8	25

accumulated during the short bouts of work to permit the collection of an adequate sample immediately after its cessation, the subject remained seated on the wheel and the blood pressure was observed at 60 second intervals for twenty minutes after the ride. Urine sample 2 was then taken. All urine samples were centrifuged and tested quantitatively for albumin, using the sulphosalicylic acid turbidimetric method (Kingsbury, Clark, Williams and Post, 1926).

Subject E. L. did a short, rapid bout of exercise on ten different days. Table 2 is a record of her pulse pressure findings during the immediate post-exercise period, and of the quantity of albumin present in the urine twenty minutes after the termination of the exercise.

All but one of the pre-exercise urine samples showed albumin in traces,

never exceeding 6 mgm./100 cc. Because of the persistence of these, complete kidney function studies were made. The blood NPN was 32 mgm./100 cc., very slightly above the usual limits of normal, but the PSP, urea concentration and Mosenthal tests conformed to standard. The negative phase in pulse pressure appeared after seven of the rides but every post-exercise urine sample contained relatively large quantities of albumin. It is of especial interest to note that following the three rides which failed to produce a post-exercise subnormal fall in the amplitude of the pulse pressure during the first twenty minutes after the cessation of exercise, an average of 40 mgm. of albumin was contained per 100 cc. of urine, in comparison with an average of 30 mgm./100 cc. in the urine samples collected after the seven rides which did induce an immediate negative phase.

TABLE 3

Showing the relationship of post-exercise albuminuria to the depth and duration of the negative phase within twenty minutes after a series of bicycle ergometer rides of the following speed, duration and severity, carried on by ten different subjects

Field current—3.2 amperes. Load terminal voltage—3.4 volts. Pedalling rate—averaged 92 R.P.M. Duration of ride—3 to 5 minutes. Rate of working—697.88 kgm.m/min.

	SUBJECT									
	DK	JK	MK	MB	DT	MM	DC	FH	KT	RP
Negative phase in pulse pressure during first 20 minutes post-exercise:										
Duration in minutes.....	2	4	9	11	11	12	12	13	13	13
Maximal depth in mm. Hg.....	2	6	10	12	12	26	28	12	12	16
Albumin in mgm./100 cc. of urine, 20 minutes after cessation of the exercise,	95	95	15	90	85	40	10	35	3	3

The average albumin in the urine which was collected after the three rides which showed the most prolonged negative phase equalled only 14.3 mgm./100 cc., being inversely related to the pulse pressure phenomenon.

A series of different subjects rode the ergometer with load and speed conditions similar to those which had invariably induced post-exercise albuminuria in E. L. The average subject found the load light, but the rate of pedalling was too high to be maintained steadily without great effort. Table 3 gives the essential findings.

The bout of work was within the functional ability of six of the subjects. One completed the ride but had great difficulty doing so because of incoordination at so high a pedalling rate. Two subjects found the muscular exertion so severe that they were forced to stop at the end of three minutes,

one complaining of palpitation, the other of dyspnea. Four minutes of pedalling at these speed and load conditions once brought on perioral blanching, then extreme pallor, severe dyspnea with shallow sighing respirations and a feeble irregular pulse. From the above evidences we may conclude that the bout of work was approximately as severe as could be tolerated by the particular group acting as subjects.

Post-exercise albuminuria occurred in every case, the amount of albumin averaging 47 mgm./100 cc. of urine. A negative phase also appeared in every case, on the average, ten minutes after the cessation of the ride. The mean negative phase of the group as a whole was relatively prolonged and deep. There was, however, no definite relationship between the appearance of albuminuria and the magnitude of the immediate post-exercise reduction in the amplitude of the pulse pressure to a level below the pre-exercise normal.

TABLE 4

Showing the amount of albumin present in urine samples collected 20 minutes after bouts of equivalent work performed at high and low speeds

Load terminal voltage—3.4. Kgm.m/min.—697.88. Duration of ride—5 minutes

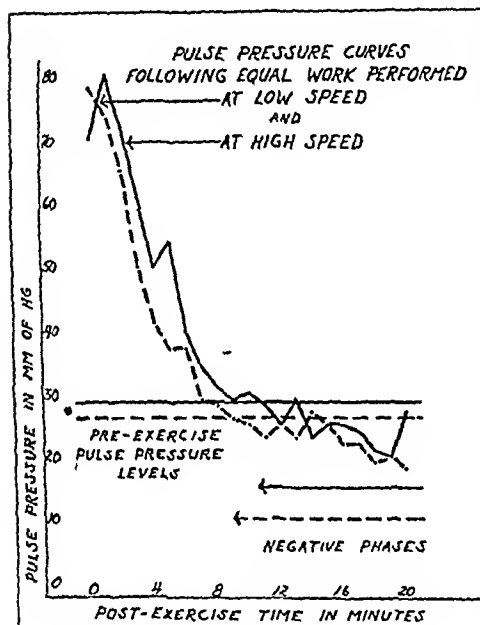
FIELD CURRENT	PEDALLING RATE	ALBUMIN IN URINE					
		DT	MM	JK	EL	MB	Average
<i>amperes</i>	<i>r.p.m.</i>	<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>	<i>mgm./100 cc.</i>	
3.2	94	85	40	95	55	90	73
5.2	65	0	2	6	10	4	4.4

Five subjects of the above series, who had shown relatively large amounts of albumin after the bout of exercise carried on for a short time at high speed, did an equivalent amount of work in an identical period of time with a heavy load at low speed. Table 4 contains the albumin findings.

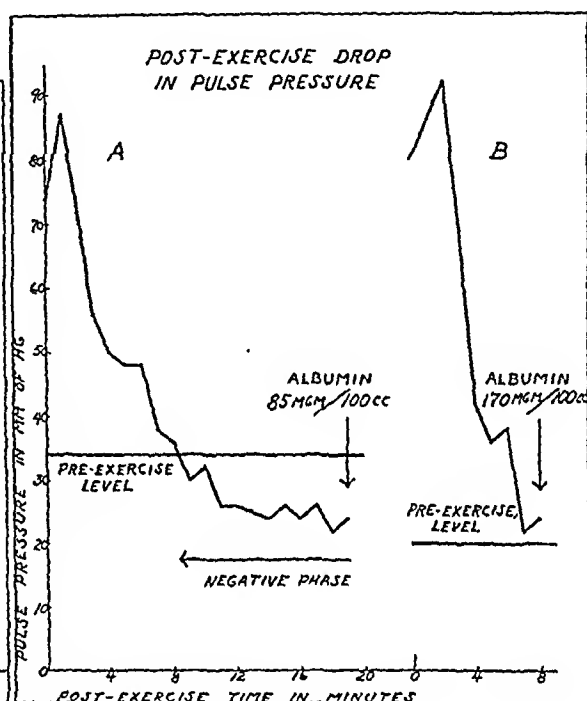
It is to be noted that a simple change in the field current was the only manipulation necessary for the establishment of the desired load and speed conditions. As long as the voltmeter needle was held at the point maintained during the fast ride, the energy input remained constant, the pedalling rate necessary to keep the needle at this point being automatically reduced with the increase in field current.

The subjects all showed relatively large amounts of albumin in the urine after the rapid piece of exercise, and the same piece of work done at low speed was followed by very small amounts. The depth and duration of the reduction in the amplitude of the pulse pressure was approximately the same after both bouts of exercise, although the albumin findings varied so markedly. Graph 4 clearly demonstrates the lack of an etiological relationship between the albuminuria and the pulse pressure under the speed and load conditions studied.

Graph 5 presents one more evidence of a lack of causal relationship between the albuminuria following short, violent exercise of speed and the post-exercise fall in pulse pressure. Subject D. T. had no albumin in the pre-exercise urine samples, but showed consistent albuminuria following various types of exercise, both experimental bouts of work and sport participation. She was able to collect adequate urine samples at relatively short intervals of time. Eight minutes after the cessation of work and



Graph 4



Graph 5

Graph 4. Showing the lack of relationship between albuminuria and the pulse pressure after equivalent bouts of work.

Graph 5. Showing the absence of an etiological relationship between the post-exercise negative phase and albuminuria. Rides A and B were identical. One urine sample was collected during the period of low pulse pressure, the other before the onset of the negative phase. Both contained albumin.

before the onset of the negative phase in pulse pressure, an abundance of albumin appeared in the urine.

Discussion. We have seen that the duration of a piece of exercise and the absolute external work done may remain constant while the speed varies. Under such conditions more energy is required to perform the rapid work than the slow because of the greatly increased internal frictional loss (Hill, 1927). The amount of oxygen required during exercise at high

speed is greater than that at low and when the response of the cardiovascular and respiratory systems is such that the intake exactly balances the requirement, the subject is in the steady state and lactic acid remains at a constant value. As the speed of work is increased and the oxygen requirement goes up, lactic acid accumulates in the blood. (Hill and Lupton, 1923.) Fletcher and Hopkins (1907) had demonstrated that lactic acid production in isolated muscle is due to oxygen want. Feldman and Leonard Hill (1911) came to the same conclusion after a study of the effect of oxygen inhalation upon the production of post-exercise urinary lactic acid in man. The lactic acid in the blood at rest is equal to 10 to 20 mgm./100 cc. (Long, 1924). After severe exercise it increases. In 1909 Ryffel found that the post-exercise excess of lactic acid after a short violent bout of work does not disappear from the urine until 30 minutes after the cessation of the exercise, a still longer period of time being necessary for the return of the blood lactic acid level to normal. In 1923 Barr, Himwich and Green found marked changes in the acid-base equilibrium of the blood after short periods of heavy muscular exercise; the blood became less alkaline, the arterial CO_2 was reduced, the CO_2 -combining capacity diminished, and lactic acid concentration increased. They believed these changes to be dependent upon the relative rates at which lactic acid is produced and removed. The CO_2 -combining capacity of the blood only gradually returned to normal and recovery was not yet complete 50 minutes after the termination of muscular exertion. Ryffel (1909) observed that the acidity of the urine definitely increased after short violent bouts of exercise. In his experimental studies on nephritis, Fischer (1912) induced albuminuria in rabbits by the continuous intravenous injection of hydrochloric acid. The urine also contained red blood corpuscles, epithelial cells and casts. Post and Thomas (1923), studying another non-nephritic albuminuria, found that every case of orthostatic albuminuria, with rare exceptions, could be made albumin-free by neutralization or mild alkalization of the urine, an etiological relationship existing between the acidity of the urine and the permeability of the renal tissue.

SUMMARY

1. Two types of post-exercise albuminuria exist. In addition to that occurring a relatively long time after the cessation of moderate, prolonged and steady exercise, another appears during long bouts of rapid and exhausting work or shortly after the termination of brief, violent exercises of speed.

2. The albuminuria occurring during exercise or shortly after its cessation is unrelated to the concomitant variations in pulse pressure, but bears an etiological relationship to the speed of doing work, occurring only after violent and rapid muscular exertion.

3. The findings may be explained by the hypothesis that exercise of speed brings about a generalized systemic increase in acidity which alters the permeability of the renal tissue to blood proteins, in consequence of which albumin appears in the urine.

BIBLIOGRAPHY

- BARR, D. P., H. E. HIMWICH AND R. P. GREEN. 1923. *Journ. Biol. Chem.*, lv, 495, 537, 539.
- FELDMAN, I. AND L. HILL. 1911. *Journ. Physiol.*, xlii, 439.
- FISCHER, M. H. 1912. *Nephritis*. John Wiley & Sons, New York.
- FLETCHER, W. AND F. HOPKINS. 1907. *Journ. Physiol.*, xxv, 247.
- HILL, A. V. 1927. *Muscular movement in man*. McGraw-Hill Book Company, New York.
- HILL, A. V. AND H. LUPTON. 1923. *Quart. Journ. Med.*, xvi, 135.
- KINGSBURY, F. B., C. P. CLARK, G. WILLIAMS AND A. L. POST. 1926. *Journ. Lab. Clin. Med.*, xi, 1.
- LONG, C. N. H. 1924. *This Journal*, lviii, 455.
- LOWSLEY, O. S. 1911. *This Journal*, xxvii, 446.
- RYFFEL, J. H. 1909. *Journ. Physiol.*, xxxix, *Proc. Physiol. Soc.*, p. xxix.

HEMODYNAMICS OF ARTERIOSCLEROSIS

INFLUENCE OF CHANGE OF COEFFICIENT OF VOLUME ELASTICITY ON CIRCULATION

GEORGE FAHR, JAY DAVIS, ARTHUR KERKHOF, PHILIP HALLOCK AND ELLIS GIERE

From the Department of Medicine, University of Minnesota

Received for publication April 18, 1932

In a previous publication (1) we have shown that the total energy consumption of the heart decreases slightly when the coefficient of volume elasticity of the large artery system is increased many times (10) provided that the change in coefficient of volume elasticity is not accompanied by a change in lumen of the large artery system. It was also shown that the external work of the left ventricle is not increased, that the diastolic blood pressure falls more than the systolic rises, that the coronary flow is slightly decreased, and that the volume flow in the systemic system does not decrease when the rigidity of the arterial system is increased to many times the normal.

In our previous work we brought about a decrease in the coefficient of volume elasticity by introducing air chambers along the large arterial system made of glass. The "effective" bore of the system remained the same when the large artery system was connected to the air chambers. In consequence of this there was no difference in the arterial resistance in the two conditions of rigidity. In arteriosclerosis of the large artery system there are static and dynamic changes of bore which must be considered. Static changes are widening of the lumen due to passive stretching of the walls and narrowing of the lumen due to intima thickening. Dynamic change of lumen is due to increase in diameter consequent upon increase of blood pressure within the tube in going from diastolic to systolic pressure during each heart cycle. The static changes can be easily evaluated. Any increase in bore reduces the resistance to blood flow approximately as the inverse ratios of the fourth powers of the diameters.¹ A decrease in bore increases the resistance to blood flow as the fourth power of the decrease in diameter of the lumen of the vessel.

¹ Poisseuille's law does not hold exactly but is a fair approximation and describes the effect of lumen change on resistance to flow well enough for the purpose of this discussion.

A rough calculation will show that the dynamic change in lumen is probably of little consequence to blood flow in the large arterial system. Let us assume that we have two tubes of the same diameter at 80 mm. Hg internal pressure. The one tube is of glass and therefore does not enlarge when the internal pressure is raised. The other tube has the same coefficient of volume elasticity as the human large arterial system. The resistance of this tube to blood flow must decrease approximately 14 per cent when the blood pressure within the tube increases from the normal diastolic to the normal systolic pressure because A. V. Hill (2) has shown in his determinations of the coefficient of volume elasticity of the adult human large artery system that the volume of the large arteries increases approximately 6.6 per cent when the pressure within is increased from 80 mm. Hg to 120 mm. Hg.² But the diameter of the blood vessel is not continuously that of systolic pressure. The vessel widens from its diastolic diameter to its systolic and then back to the diastolic diameter during each pulse wave. Using the best optic manometer curves of aortic pressure for calculation, it can be shown that the mathematical "mean" increase in diameter of the vessel lumen is less than 70 per cent of the maximum systolic increase in diameter. In other words, the widening of the large arterial system during a complete heart cycle reduced the "effective" resistance of the system not more than 10 per cent. If we assume that the resistance of the large arterial system is 25 per cent of the whole vascular resistance, then we see that the total reduction in resistance to blood flow due to widening of the lumen of the large artery system during systole is only about 2.5 per cent of the total resistance; an almost negligible decrease in resistance.

Despite the results of the foregoing calculation we know that it would be impossible to convince many physiologists and clinicians that a great increase in rigidity of the large artery system probably would not increase the work of the left ventricle unless the proof was experimentally demonstrated. The idea of E. A. Weber (3) that the work of the heart is greatly reduced by the substitution of elastic tubes for rigid tubes is firmly fixed in the minds of most physiologists and clinicians. In order to prove experimentally that the work of the heart is not increased appreciably by a great increase in rigidity of the large arteries and in order to study other factors of the circulation when the coefficient of volume elasticity is increased, the following experimental method was devised. See figure 1.

The heart-lung preparation of Starling was used in connection with an artificial large artery system whose coefficient of volume elasticity could be changed at will from values about half that of the normal adult human large artery system to values nearly ten times that of the normal adult human large artery system. This large artery system consisted of a piece

² This calculation of decreased resistance from increase in lumen is based upon Poiseuille's equation and is only approximate.

of thin walled rubber tubing of diameter about 12 mm. and length 100 cm. kindly constructed by the research department of the Goodrich Rubber Company³ so as to have a coefficient of volume elasticity about half that of the normal adult large artery system. This tube was enclosed within a heat insulated lead pipe of internal diameter 35 cm. The lead pipe had five stopcocks inserted at equal intervals along its wall. These stopcocks were connected to glass jars by rubber tubing. The rubber tubing within the lead pipe was filled with physiological salt solution under a pressure of about 25 cm. H₂O and clamped off. Then water was filled into the lead pipe, care being taken to exclude all air bubbles. The stopcocks were then closed and the rubber tube connected at one end to the aortic cannula of the heart-lung preparation and at the other end to the artificial resistance of the system and blood allowed to flow from the heart through the rubber tube, and artificial resistance into the venous reservoir of the heart-lung preparation. With the stopcocks closed the coefficient of volume elasticity of the large artery tube is that of the water mantle, i.e., extremely high rigidity; when the stopcocks are opened to the air the coefficient of volume elasticity of the tube is that of the rubber tubing or approximately half the normal. Coefficients of any desired value between half that of the normal artery and that of water could be obtained when the stopcocks were connected to bottles of various capacity filled with air at atmospheric pressure and with the cocks open. Thus it is possible to quickly vary the rigidity of the artery from a value half that of the normal large artery system of the adult to any value desired up to that of an almost incompressible fluid by simply closing and opening five stopcocks in the lead jacket. In carrying out the experiments the stopcocks were closed as nearly as possible at the end of diastole in order that the diameter of the tubing in the state of increased rigidity would be as nearly as possible that of the diastolic condition in the less rigid state. That we succeeded in this is shown by the fact that we could repeat the process of closing and opening stopcocks many times with nearly perfect repetition of the volume curves of the ventricles and nearly perfect repetition of the blood pressure curves! The large artery system is connected to the arch of the aorta by a cannula of 9 mm. internal bore inserted into the descending aorta up close to the aortic valves. At the other end it is connected by a glass tube 10 cm. long and bore 3.5 mm. with an artificial resistance for regulating blood pressure to any desired value. The artificial resistance is connected to the venous reservoir as in figure 1. The water mantle around the large artery is filled with water at about 45°C. This cools to a temperature such as to keep the blood entering the superior vena cava fairly constantly at about 32 to 35°C. after being put into the lead jacket. The resistance of the large artery

³ We wish to thank Dr. J. W. Schade for his kindness in having tubing constructed according to our specifications by the Goodrich Rubber Company.

system in our setup at the pressures we worked with was 10 to 12 per cent of the whole vascular resistance. A mercury manometer was connected to the aortic cannula for registration of blood pressure. In a few instances a Wiggers optical manometer was used to get more accurate blood pressure curves. A Henderson cardiometer was inserted over the heart to measure the volume of the ventricles. This cardiometer was connected by copper tubing to a Wiggers segment capsule for recording the changes in volume

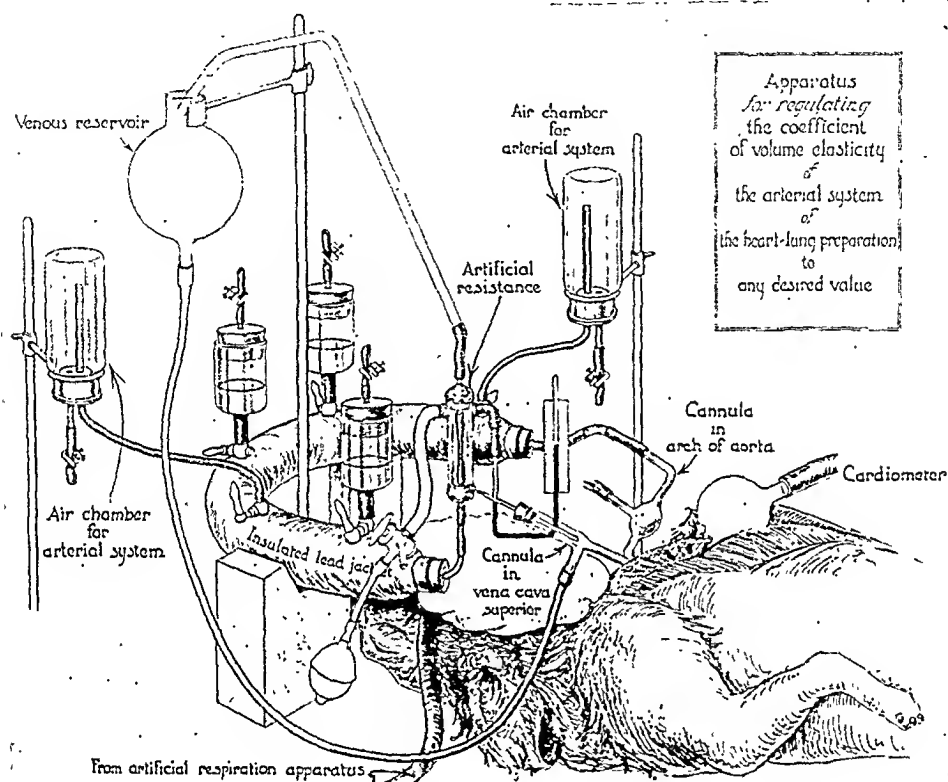


Fig. 1. Apparatus for studying effects of arteriosclerosis upon the circulation in the heart-lung preparation. In all our experiments, the bottles connected to the stopcocks were kept open to the atmosphere so that with the stopcocks opened, coefficient of volume elasticity of the rubber tubing within the lead pipe and water mantle is that of the rubber tubing.

of the ventricles. The cardiometer tracings were calibrated by injecting 20 cc. of air into the system through a side tube and the corresponding descent of the lever marked on the drum. Time was measured in seconds on the drum by a Jaquet chronometer. The lower position of the chronometer line was adjusted to the 0 point of the mercury manometer and is therefore the base line of the mercury manometer. A signal marked the moment of closing or opening the stopcocks on the lead jacket, or in other

words the time of transition from arteriosclerosis to normal artery and vice versa.

The circulating minute volume, i.e., the true minute volume minus the coronary flow, was measured by means of a stopwatch graduated to 0.1 second and a measuring cylinder marked off for volumes of 150 cc. Blood

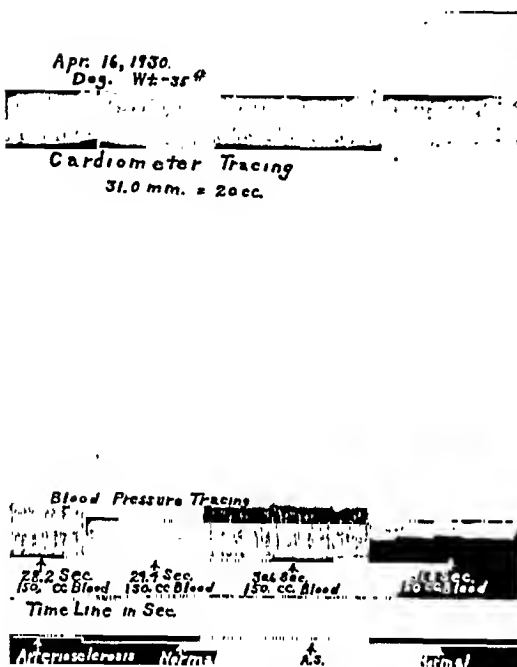


Fig. 2

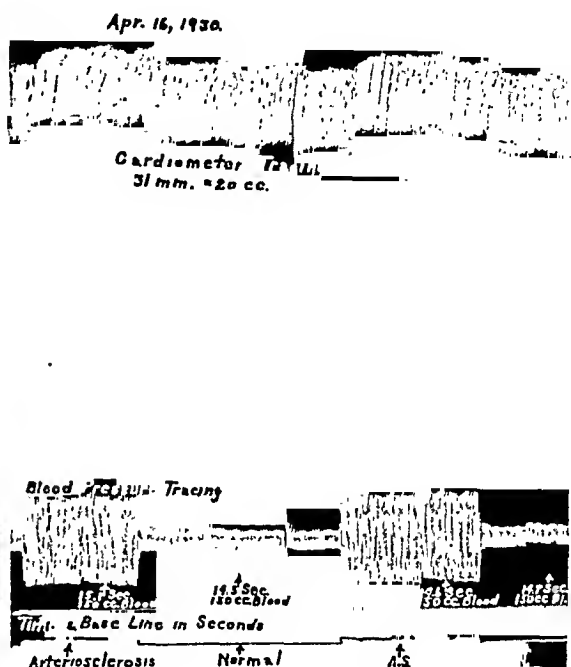


Fig. 3

Fig. 2. Tracing of experiment of April 16, 1930. Upper tracing cardiometer volume; lower tracing mercury manometer. Moment at which out-flow from venous end was measured indicated by arrows below blood pressure tracing. Time of out-flow of 150 cc. of blood marked in seconds. Upstroke of cardiometer tracing is systole; downstroke diastole. During the arteriosclerosis the diastolic volume of the heart is approximately 1.5 cc. smaller than during the normal. Pulse pressure in arteriosclerosis is approximately three times as great as in the normal.

Fig. 3. Experiment of April 16, 1930, after increasing the artificial resistance very slightly and increasing the inflow from the venous reservoir into vena cava superior. Pulse pressure in arteriosclerosis approximately four and one-half times as great as in normal. During arteriosclerosis, diastolic volume of heart is approximately 3 cc. smaller than in normal.

leaving the end of the rubber tubing and entering the venous reservoir could be diverted at will into the cylinder. The time taken to fill the 150 cc. was determined by the stopwatch. The minute flow could be measured in this way with an accuracy of ± 3 per cent.

The cardiometer was always carefully placed around the ventricles and

care taken to avoid leaks. It was impossible for us to determine the accuracy of our measurements of stroke volume from the cardiometer tracings but we believe the error was not large. The error in stroke volume determines the error in the minute volume determination because the minute volume is stroke volume times the number of heart beats per minute. The latter can be accurately determined. Coronary flow was determined

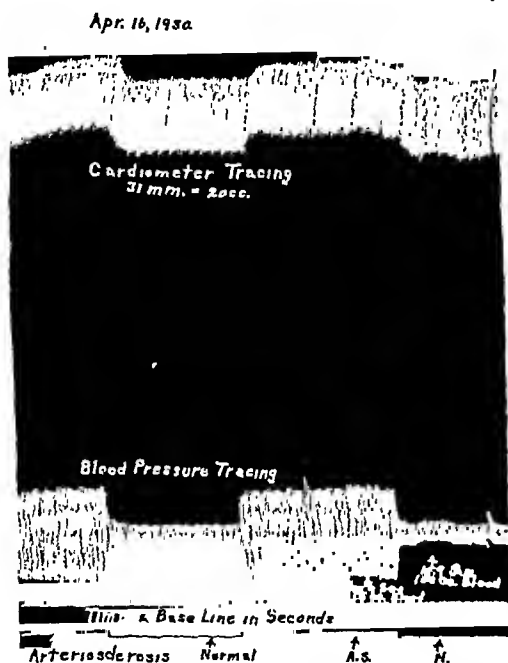


Fig. 4

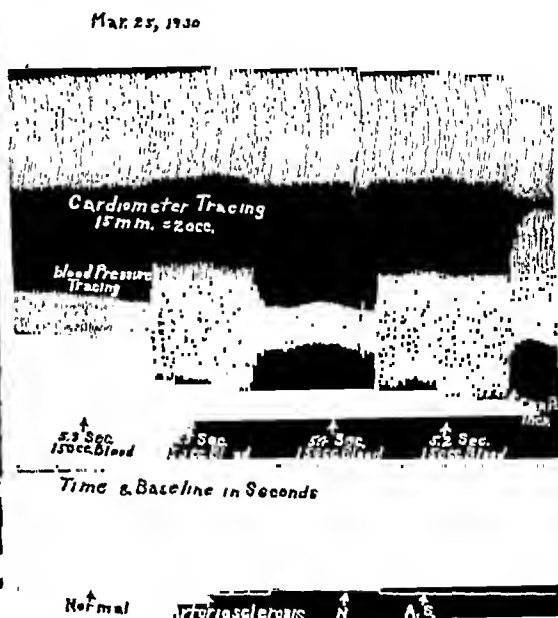


Fig. 5

Fig. 4. Experiment of April 16, 1930. Same as figure 3 excepting that tracing was taken ten minutes later. Pulse pressure is approximately four times as great in arteriosclerosis. During arteriosclerosis, diastolic volume of the heart is approximately 3.5 cm. less than in normal.

Fig. 5. Experiment of March 15, 1930. In this experiment in which the minute volume is very large and the blood pressure moderately elevated, we see that the diastolic volume of the heart is approximately 3 cc. smaller in arteriosclerosis than in the normal. Pulse pressure in arteriosclerosis is approximately 2.7 times as large as in the normal and the circulating volume approximately the same in both conditions.

by subtracting the relatively accurate circulating volume from the minute volume of the left ventricle as determined from half the cardiometer stroke volume times the heart beat. The absolute error in this determination is the error in the cardiometer stroke volume. We believe that the minute volume determination is accurate to 10 per cent. The

diastolic position of the cardiometer tracing would sometimes stay constant for a half-hour or longer provided there was no change made in the inflow into the superior vena cava and no change made in the artificial resistance and provided the temperature remained constant. On the other hand the coronary flow which is determined by a difference in quantities many times larger is not nearly as accurate (in percentage) as the stroke volume.

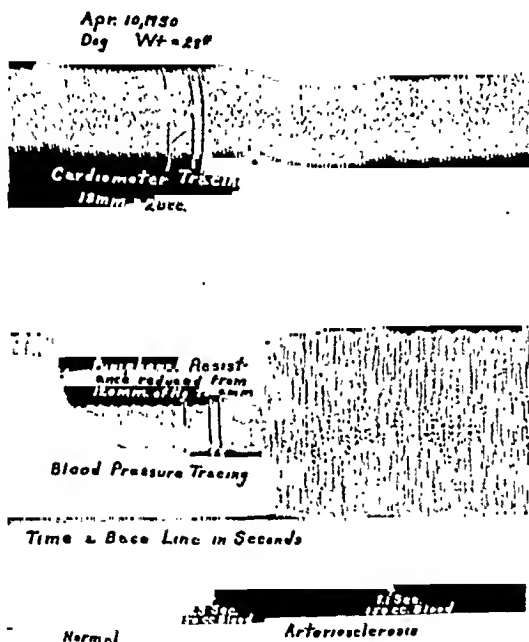


Fig. 6. Experiment of April 10, 1930. Working with a very low blood pressure and a very high stroke output, we see that during arteriosclerosis there is a dilatation of the heart of approximately 2 cc. The flow through the coronaries with the stroke volume is very much less during arteriosclerosis; at the same time the flow through the rest of the arterial system is the same as in the normal, the result being a very much reduced flow through the coronary arteries with an approach to the hypodynamic condition.

In determining the coefficient of volume elasticity a clamp was placed on the rubber tubing connecting the artificial resistance to the glass tube leading to the venous reservoir, thus closing the system at the venous end of the artificial resistance. The whole system was kept filled with blood and a record syringe filled with saline was connected to the aortic end of the aortic cannula; 2 cc. amounts of saline were injected into the system with the stopcocks of the lead jacket closed (arteriosclerosis) and the

corresponding rises in pressure recorded on the drum by the mercury manometer. With the stopcocks of the lead jacket open (normal artery) 20 cc. amounts of saline were injected into the aortic cannula and the corresponding rises in pressure recorded on the drum by the mercury manometer. We have expressed rigidity or coefficient of volume elasticity in terms of 1 per cent increase in volume to corresponding rise in millimeters mercury pressure. It will be seen that what we determined by this calibration is the coefficient of volume elasticity of the whole system from the heart to the venous end of the artificial resistance with the mercury manometer in the system. This value is much less than the coefficient of volume elasticity of the tube within the lead jacket when the stopcocks are closed because the artificial resistance adds a tube of very low rigidity and the mercury manometer itself is a tube of even lower rigidity. When the stopcocks are opened (normal) the elastic effects of the artificial resistance and the mercury manometer make very little change because they both have low coefficients of elasticity of the same order of magnitude as the rubber tubing in the jacket and moreover, their volume is very small in comparison to the volume of the tubing within the lead jacket. In determining the volume of the system, all the fluid in the system including that in the artificial resistance, that within the tubing in the jacket, the content of the arterial cannula and the fluid in the connections of the mercury manometer were measured. It can be said that the values determined therefore are very much lower than the value of the coefficient of volume elasticity for the large artery system alone in the condition of great rigidity or arteriosclerosis and are just a little high for the value of the coefficient of volume elasticity of the large artery tubing in the normal condition. The error in our experimentally determined coefficients of volume elasticity due to the fact that the tip of the aortic cannula reached only to within 1.5 or 2 cm. of the root of the aorta is of such an order of magnitude that it can be neglected.

We shall consider that the diastolic volume of the heart is an index of the total energy consumption of the heart as first proved by Starling and Vischer (4) and that as a first approximation the product of minute volume and mean blood pressure is equal to the external work of the left ventricle. The energy consumption of the heart increases if the heart dilates and decreases if the heart gets smaller, provided the inherent contractile property of the heart muscle remains the same. Everything else being equal, the external work of the left ventricle may be looked upon as being increased if the diastolic volume of the heart increases and it may be looked upon as being decreased if the diastolic volume of the heart decreases. Unchanged diastolic volume means unchanged amount of external work performed by the heart.

Experiment of April 16, 1930. Calibration of elastic coefficient in terms of percentage increase of arterial system to millimeter of mercury increase in pressure or mm. Hg increase in pressure

$$\frac{\% \text{ increase in volume}}{\text{mm. Hg increase in pressure}} = \text{"effective" elastic coefficient or rigidity of arterial system.}$$

Stopcocks closed (arteriosclerosis)

2 cc. fluid injected into system of 240 cc. capacity gives a rise of 48 mm. Hg in pressure within this system.

$$\frac{2}{240} = 0.83 \text{ per cent increase in volume for 48 mm. Hg.}$$

One per cent increase in volume for 58 mm. Hg.

A. V. Hill's normal artery between 80-120 mm. Hg showed 1 per cent increase in volume for 6 mm. Hg.

In this arterial system with stopcocks closed there is a rigidity $9.5 \times$ the "mean" normal artery of A. V. Hill at normal pressure; i.e., an extremely severe arteriosclerosis.

Stopcocks open (normal)

20 cc. fluid injected into arterial system gives a rise of 28 mm. Hg.

$$\frac{20}{230} = 8.7 \text{ per cent increase in volume for 28 mm. Hg pressure.}$$

1 per cent increase in volume for 3.2 mm. Hg.

This rigidity is approximately $\frac{1}{2}$ that of A. V. Hill's "mean" normal artery between the pressures of 80-120 mm. Hg.

Rigidity with stopcocks closed is 18 times rigidity with stopcocks open.

<i>Stopcocks open</i> (Normal)			<i>Stopcocks closed</i> (arteriosclerosis)	
Sys. B.P.....	56	Mean B.P. 49.5	66	Mean B.P. 47
Dias. B.P.....	43		28	
Stroke Vol.....	5.6 cc.	Figure 2.	5.2 cc.	
Minute Vol.....	504 cc.	Heart rate 90	468 cc.	
Circul. Vol.....	299 cc.		294 cc.	
Coronary flow.....	205 cc.		174 cc.	

Inflow increased. Artificial resistance slightly increased.

Sys. B.P.....	58	Mean B.P. 51	81	Mean B.P. 50
Dias. B.P.....	44		19	
Stroke Vol.....	9.0 cc.	Figure 3.	8.7 cc.	
Minute Bol.....	810 cc.	Heart rate 90	783 cc.	
Circul. Vol.....	615 cc.		615 cc.	
Coronary flow.....	195 cc.		168 cc.	

ten minutes later

Sys. B.P.....	58	Mean B.P. 50.5	80	Mean B.P. 49
Dias. B.P.....	43		18	
Stroke Vol.....	8.4 cc.	Figure 4.	8.0 cc.	
Minute Vol.....	756 cc.	Heart rate 90	720 cc.	
Circul. Vol.....	565 cc.		565 cc.	
Coronary flow.....	191 cc.		155 cc.	

Stroke Vol. = Output of one ventricle. Obtained by multiplying the height of cardiometer curve in millimeters by 20 and dividing by 2 times the increase in height of cardiometer tracing when 20 cc. are forced into the cardiometer system from a record syringe connected to the system.

Minute Vol. = Total output of left ventricle calculated from stroke volume multiplied by heart rate.

Circul. Vol. = Volume of flow as measured with stopwatch and calibrated measuring cylinder on blood flowing out of venous system into venous reservoir.

With stopcocks closed, i.e., in arteriosclerosis, the diastolic volume is less, showing that the energy consumption of the heart is less in arteriosclerosis. This is due to the decrease in external work of the left ventricle largely. The arithmetical mean pressure falls slightly and the minute

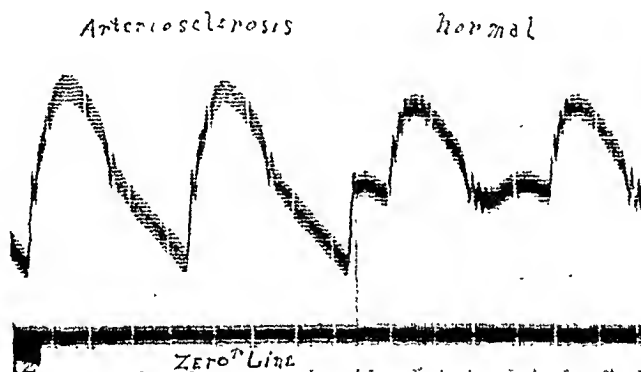


Fig. 7. Optical records of blood pressure in the aorta. First two curves are arteriosclerosis, systolic blood pressure being 97 and diastolic blood pressure 28. The last two curves are taken from the normal artery, systolic blood pressure is 86 and diastolic blood pressure 56. The aortic pressure curves taken with optical manometers on other days did not always show the secondary wave in diastole, but the fall of pressure during diastole was always very slight in the normal because of the low coefficient of elasticity of the artery.

volume also. The external work of the heart measured by their product is definitely less. In fact blood pressure curves taken with the optical manometer show an even greater reduction in diastolic and arithmetical mean pressures (see fig. 7). The coronary flow is always definitely less in arteriosclerosis.

Experiment of March 25, 1930. Calibration of elastic coefficient in terms of percentage increase in volume of arterial system to millimeter of mercury increase in pressure.

Stopcocks closed (arteriosclerosis)

2 cc. fluid injected into system of 260 cc. capacity gave a rise of 40 mm. Hg in pressure within this system.

1 per cent increase in volume for 52 mm. Hg or a rigidity $8.6 \times$ the "mean" normal artery of A. V. Hill.

Stopcocks open (normal)

20 cc. fluid injected into system of 290 cc. capacity gave a rise of 20 mm. Hg in pressure within this system.

1 per cent increase in volume for 2.9 mm. Hg or a rigidity $\frac{1}{2}$ the "mean" normal artery of A. V. Hill.

The rigidity with stopcocks closed is 18 times the rigidity with stopcocks open.

<i>Stopcocks open</i> (Normal)			<i>Stopcocks closed</i> (arteriosclerosis)
Sys. B.P.	120	} Mean B.P. 105	140
Dias. B.P.	90		58
Stroke Vol.	26.0 cc.	Figure 5.	24.5 cc.
Minute Vol.	2028 cc.	Heart rate 78	1911 cc.
Circul. Vol.	1665 cc.		1635 cc.
Coronary flow	363 cc.		276 cc.

With the minute volume very large we see that the diastolic volume of the heart decreases in arteriosclerosis corresponding to a decrease in energy consumption of the heart. The external work of the heart also decreases as measured by the product of minute volume and arithmetical mean pressure.

Experiment of April 10, 1930.

Stopcocks closed (arteriosclerosis)

2 cc. fluid injected into system of 200 cc. capacity gives 40 mm. of mercury increase within this system.

1 per cent increase in volume for 40 mm. Hg or a rigidity 6.6 times the rigidity of A. V. Hill's normal arterial system.

Stopcocks open (normal)

20 cc. fluid injected into system of 240 cc. capacity gives 24 mm. Hg rise in pressure within this system.

1 per cent increase in volume for 2.8 mm. Hg or a rigidity $\frac{1}{2}$ that of A. V. Hill's normal.

The rigidity with stopcocks closed is 14 times that with stopcocks open.

<i>Stopcocks open</i> (Normal)			<i>Stopcocks closed</i> (arteriosclerosis)
Sys. B.P.	82	} Mean B.P. 66	124
Dias. B.P.	50		0
Stroke Vol. ...	1611 cc.	Figure 6.	15.2 cc.
Minute Vol. ...	1207 cc.	Rate of heart 75	1140 cc.
Circul. Vol. ...	1084 cc.		1110 cc.
Coronary flow	123 cc.		30 cc.

In this experiment we see a dilatation of the heart of 4 cc. in the condition of arteriosclerosis. This is the only experiment we have showing a dilatation of the heart in arteriosclerosis. The cause for this is to be found in the inadequate coronary flow during the arteriosclerotic period of the experiment. Though the external work of the heart is decreased in arteriosclerosis, yet the heart dilates because it is

becoming hypodynamic due to inadequate coronary blood flow. The work of the left ventricle in this heart is 0.94 kilogram-meter per minute and reckoning the work of the right ventricle at $\frac{1}{3}$ of this, the external work of the heart is 1.13 gram meters per minute or 0.028 calorie which is equivalent to 5.8 cc. of oxygen consumption. If we assume the mechanical efficiency to be 40 per cent, then the total oxygen consumed by heart to perform this work will be 14.5 cc. Thirty cubic centimeters of arterial blood only contains 6 cc. of oxygen. Therefore there is insufficient oxygen supply for the work of the heart muscle assuming that the coronary flow as determined above is correct.

In figure 7 we show aortic pressure curves recorded by a Wiggers optical manometer at 10 feet distance. These curves are very accurate records of the blood pressure relations in arteriosclerosis and in the normal. The first two aortic pressure curves were taken with stopcocks closed (arteriosclerosis) and a coefficient of volume elasticity eight times that of the normal adult large artery system. The last two curves give the pressure relations in the aorta when the stopcocks were open (normal) and the coefficient of volume elasticity was half that of the normal adult large artery system. Calibration of the optical manometers show

<i>Arteriosclerosis</i>		<i>Normal</i>	
Sys. B.P.	97	86	Mean B.P.
Dias. B.P.	28	56	71

By integration of the pressure curve in arteriosclerosis and the pressure curve in the normal artery and dividing by the abscissa, we have determined the "mathematical" mean pressures in the two conditions.

"Mathematical" mean pressure for one whole cycle

Arteriosclerosis.....	62
Normal.....	66

Blood is thrown into the aorta only from the beginning of the systolic rise to the diastolic notch and therefore the mathematical mean pressure during this period times the minute volume is a better index of the work of the left ventricle than the minute volume times the mathematical mean pressure for a whole pulse cycle. By integration we have determined this as approximately the same in both conditions.

Mathematical mean pressure for systole

Arteriosclerosis.....	79
Normal.....	78

If we wish to get an idea of the magnitude of the blood pressure effective in causing flow through the coronary arteries, it is more nearly correct to integrate the pressure curves from the diastolic notch to the end of diastole for most of the flow in the coronary arteries occurs during diastole. We have done this for the above curves and find for the true mean pressure during diastole:

Arteriosclerosis.....	41
Normal.....	52

The minute volume through the coronary arteries should be proportional to these figures. As a matter of fact the coronary flow in arteriosclerosis and in the normal, in our experiments, is very closely proportional to these figures, excepting the experiments of April 10th when the coronary flow during arteriosclerosis was only about 25 per cent of that in the normal system.

This investigation proves conclusively that a very great increase in the coefficient of volume elasticity of the large artery system does not increase the external work of the left ventricle nor increase the energy consumption of the heart; rather it leads to a small decrease in both these magnitudes. Moreover the minute volume in the arteries exclusive of the coronary arteries is not decreased in severe arteriosclerosis.

The only adverse factor that our investigation brought to light was a decreased coronary flow; in one instance the coronary flow was so small that the contractile property of the heart muscle decreased. The cause of this decreased flow is to be found in the lowered mathematical mean pressure during diastole. The systolic pressure rises in arteriosclerosis but the diastolic pressure decreases more than the systolic rises. More especially the mean pressure during diastole decreases greatly in the arteriosclerotic condition. The normal artery tends to hold up the pressure after the closure of the semilunar valves because the energy stored up in the stretched elastic walls tends to be transformed into pressure during diastole. This tendency to maintain blood pressure at a high level during diastole is very probably a very important consequence of the low coefficient of volume elasticity of the large artery system of man. The vagus effect upon the coronary arteries of the dog is destroyed in the heart-lung preparation so that we probably have a maximal bore in these vessels in this preparation as a rule. If the bore should be reduced let us say by intima thickening as in coronary arteriosclerosis or by any other mechanism, then with an increase in rigidity in the large arteries, the flow through the coronaries might be lowered to a point where the muscle would lose its inherent contractile properties.

Some observers (5), (6) have claimed that the flow through a tube of small coefficient of volume elasticity is very much greater than the flow through a tube of greater rigidity or that the flow through an organ is greater when using a pulsating pressure source instead of a constant pressure source. These observers have used experimental methods which throw little light on the problems of hemodynamics when the rigidity of the large artery system alone is greatly increased. Romberg (5) compared the outflow from two pieces of tubing, one of glass and the other of very easily stretched rubber, of the same diameter under zero pressure and the

two pieces of tubing making up the whole resistance of the system, whereas the resistance of the large artery system is less than 25 per cent of the whole vascular resistance. If we should have used a glass tube of internal diameter 12 mm. and our rubber tube of diameter 12 mm. when no pressure was within the tube and applied a pulsating pressure varying from 120–80 mm. Hg the outflow from the rubber tube would have been 90 per cent greater than from the glass tube because the rubber tube would have dilated in going from 0–80 mm. Hg and again it would periodically increase its bore in going from 80 mm. Hg to 120 mm. Hg. Fleisch (6) compared the outflow from whole organs as kidney when a pulsating pressure was acting, with the outflow produced by a constant pressure equal to the mean of the pulsating pressure. Here also the outflow was greater under pulsating pressure because the small arteries and veins dilated very much under increased pressure in systole because by Poiseuille's law there must be a greater outflow with pulsating pressure. On the other hand, we have only attempted to study the circulation as modified by a change in rigidity of the large artery system, the small artery, the capillary, and the venous systems remaining unchanged. Moreover we have attempted to keep the diastolic bore of the large artery system the same in both states, allowing the diameter to increase only during systole in the less rigid state. A more extensive review and discussion of other work in this field will be given when we publish our third paper on the subject taking up the problem of circulation when the rigidity of the small arteries and capillaries is altered.

CONCLUSIONS

Provided that the diastolic diameter of the rigid artery is the same as that of the less rigid artery and the resistance of the large artery system is about 10 to 15 per cent of the whole vascular resistance:

1. The energy consumption of the heart is not increased but is slightly decreased when the coefficient of volume elasticity of the large arteries is greatly increased.

2. The external work of the left ventricle is not increased when the coefficient of volume elasticity is greatly increased, rather the external work is slightly decreased in severe arteriosclerosis of the large arteries because the "mathematical mean" pressure during systole remains nearly the same and the minute volume decreases a little.

3. The flow through the coronary vessels decreases 20–25 per cent after severe increase in the rigidity of the large arteries because the "mathematical mean" pressure falls about this much during diastole, the period during which most of the flow takes place in the coronary arteries. Under certain circumstances, it may fall so far that the inherent contractile property of the heart muscle is impaired.

BIBLIOGRAPHY

- (1) FAHR, G., J. DAVIS AND R. SPITTLER. This Journal, 1931, xevi, 426.
- (2) HILL, A. V., J. C. BRAMWELL AND A. C. DOWNING. Heart, 1923, x, 289,
- (3) WEBER, E. H. Annotationes Anat. et Physiol., programmata collecta, I.
Leipzig, 1831. Cited by TIGERSTEDT in Physiologie des [Kreislafs,
Vol. 3. p. 12.
- (4) STARLING, E. H. AND M. B. VISSCHER. Journ. Physiol., 1927, lxii, 243.
- (5) ROMBERG, E. Verh. d. Kongr. f. inn. Med., 1904, 60.
- (6) FLEISCH. Pflüger's Arch., 1919, clxxiv, 177. 1920, clxxviii, 38.

THE PASSAGE OF UREA BETWEEN THE BLOOD AND THE LUMEN OF THE SMALL INTESTINE

WALTER R. PENDLETON AND FRANCIS E. WEST

From the Department of Physiology and Pharmacology, University of Southern California School of Medicine, Los Angeles, California

Received for publication April 29, 1932

The purpose of this work was to determine whether or not urea will pass from the blood to the contents of the bowel, and to study the conditions of its passage. Twenty-one dogs were used. In some cases two and three series of experiments were run on each dog.

METHODS. Urea and ammonia determinations were made by the Folin-Wu distillation method in the earlier experiments. Later the aeration method was used. In our hands the aeration method proved more convenient and more constant in results. All final readings were made colorimetrically after Nesslerization. The urea content of the blood was found systematically higher by the Folin-Wu method which, it seems likely, is because in diluting to a definite volume before precipitation, the volume of protein precipitate is neglected. By this method the real dilution is less than the supposed, leading to high values. In the aeration method the blood was measured and treated with urease and aerated, allowing for no such error of dilution. The differences in the two methods are not great enough to interfere with their usefulness in this problem. The blank due to the urease, usually 1.7 mgm. per cent in routine determinations, and a correction due to caprylic alcohol passed over in the aeration process, were deducted in making calculations. Checks on known solutions showed these corrections to be valid.

An interesting source of error was encountered in these studies. When tap water was placed with Nessler's solution in any of various proportions there was no color change. It was therefore at first concluded that the city water contained none of the Nessler reacting substances. Later it was found that when Nessler's reagent reacted to ammonia (as in all the routine determinations) a small proportion (2 to 5 drops) of city water would cause a very serious clouding. A rather exhaustive study showed that the minerals in the city water actually caused the clouding, and that it would occur consistently, also that no clouding would occur unless at least a trace of ammonia was present. Rinsing in distilled water made glassware free from this effect, ordinarily, but it was found that occa-

sionally commercially distilled water also contained enough of the clouding substance to cause loss of unknowns. If the amount of clouding substance was very slight, then by rapidly making colorimetric determinations one might finish before the solution definitely clouded. This is hardly commendable. There was absolutely none of this trouble when a redistilled water was used for making dilutions and for rinsing.

All values for "urea nitrogen" unless otherwise specified include urea plus ammonia nitrogen.

Dogs were used under sodium amytal anesthesia. The dose was approximately 60 mgm. per kilo. In some of the work the amytal was injected into the thigh muscles. In the later experiments it was given intraperitoneally. Frequently the amytal anesthesia was supplemented by ether inhalation, usually for short intervals.

The bowel was cleaned in each dog by flushing with normal saline solution at body temperature. About four liters would be run through in slightly less than an hour in cleaning the bowel. The washings would be quite clear by this time. The clamping off effect of peristaltic waves blocked the free flow of the solution to some extent. However, a fluid pressure (elevation) of about 17 cm. generally proved sufficient to overcome such obstruction.

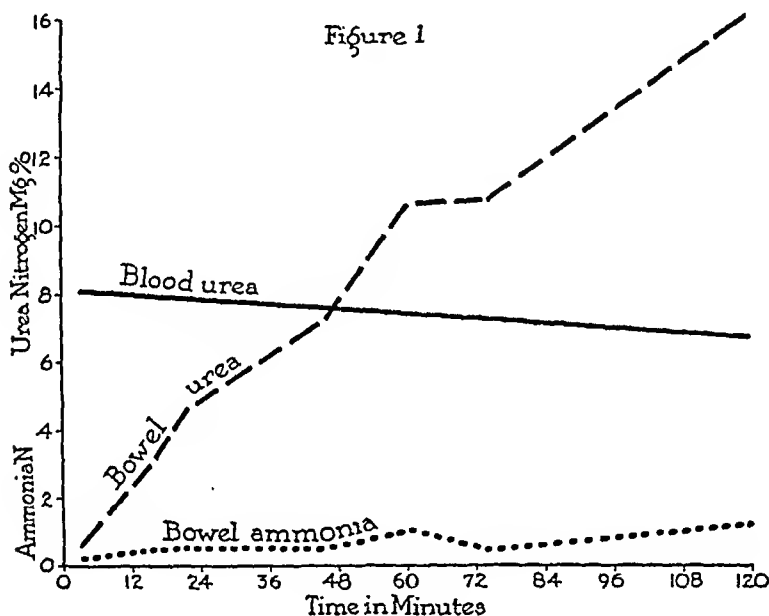
Solutions entered the small bowel through a rubber tube inserted into the middle third of the duodenum. Solutions left the small bowel through a larger rubber tube fastened into the ileum near the ileo-cecal junction. The bowel above and below this loop was tied off. Thus practically all of the small bowel was used as the loop for these tests. Samples were generally taken by inserting a Luer needle diagonally through the bowel wall each time. Generally about 8 cc. would be removed for determinations.

RESULTS. When normal saline solution was placed in the small bowel, its urea content rapidly rose to that of the blood, as is shown for a typical experiment in figure 1. In many of the animals the bowel contents showed 6 mgm. per cent of urea nitrogen in five minutes. In fifteen minutes it reached 20 mgm. per cent. This was already slightly in excess of the blood urea nitrogen.

The rate of urea rise may be expected to vary in different dogs depending on the condition of the bowel and on the degree of general depression incidental to experimental procedures and anesthesia. It is interesting and important that in practically every instance in which the experiment was continued to the point of apparent equilibrium, the bowel concentration of urea reached a figure somewhat above that of the blood plasma.

To obtain more information regarding the maximum bowel urea level at equilibrium we approached it from the opposite side. We started with a very high concentration of urea in the bowel. Using an 11 kgm. dog we placed 100 cc. of the normal saline solution containing 200 mgm. of

urea into the loop of small bowel. The concentration was equivalent to 95 mgm. per cent of urea nitrogen. The kidneys were left intact here as in all preceding experiments. One hour and fifteen minutes was allowed for establishing equilibrium. At this time the urea nitrogen in the intestine had fallen to 35 mgm. per cent and the blood urea nitrogen had risen to 23 mgm. per cent. At two hours and fifteen minutes the bowel had 32 mgm. per cent urea nitrogen and the blood 26 mgm. per cent, and the fluid was almost completely absorbed. Thus on reaching apparent equilibrium the bowel content became temporarily established at a point higher than that of the blood stream.

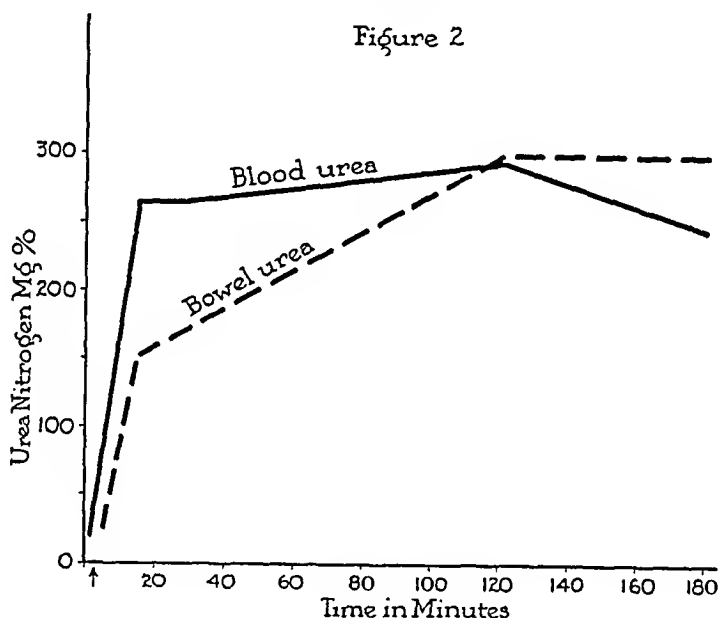


We were next concerned with the influence of a high blood urea on the equilibrium. Four dogs were used. The kidneys were removed from the body before running the tests. In each case urea was injected intravenously, allowing about 5 minutes for injection. Plain normal saline solution was used in the loop of bowel.

The results showed a rapid rise of urea in the bowel to the blood level. Figure 2 shows one experiment typical of the others. This dog received urea equivalent to 1.5 gram of urea nitrogen per kilogram of body weight. The blood urea nitrogen before injection was 16 mgm. per cent. The 15 and 30 minute specimens of blood urea were each 265 mgm. per cent. It is worthy of note that the intestinal contents reached 25 mgm. per cent in 5 minutes and 153 mgm. per cent in 15 minutes. At 2 hours the blood urea nitrogen was 290 mgm. per cent and the bowel 299. At 3 hours the blood urea had fallen to 245, while the bowel still contained 281 mgm. per cent.

One test was made on the large bowel, using normal saline in the loop, and having a normal blood urea. In 30 minutes the urea nitrogen rose to 19 mgm. per cent in the bowel. It seems that the large bowel resembles the small bowel in regard to diffusion of urea out of the blood, but our observations are not adequate to establish the equilibrium conditions.

Determinations were made on the ultrafiltrates of blood and of bowel contents. This eliminated all the protein material and gave the concentration of urea in the active fluid. The ammonia in the bowel fluid was now also determined in each case. Ammonia was not measured in the blood as a routine in the experiments, since control observations showed—and it is well known—that the ammonia content of the blood drawn under proper conditions is extremely low, too low to be a factor here. Ultra-



filtration showed that the actual corrected urea nitrogen of the bowel (ammonia deducted) might be as high as 17.7 mgm. per cent, while that of the ultrafiltrate of blood was 12.0 mgm. per cent. We are left with no explanation of our findings except that in some manner urea seems to be concentrated in the bowel.

With normal saline in the bowel there was always a continuous fluid absorption. Could it be that the urea passed through the bowel wall during absorption somewhat more slowly than the water in which it was dissolved? If the bowel wall is more permeable to water than to urea such a state of affairs could exist, but there would be a certain concentration of bowel urea at which urea would leave at a rate identical with that of the liquid in which it was dissolved. This then would be the point of equi-

librium. We would assume that if one started with no urea in the fluid within the bowel, urea would enter the bowel as through a partially semi-permeable membrane. When a concentration equal to that in the blood stream was reached, then the continued fluid absorption would further concentrate the urea to the point of dynamic equilibrium around which it would be maintained.

If this be the proper interpretation, the inhibition of water absorption should change the equilibrium concentration of urea in the bowel to a figure exactly equal to that in the blood. We tried various fluids in the bowel to get one that would not be absorbed and finally used 5 per cent $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. This maintained a constant fluid volume. It did not seem to cause difficulty from bowel irritation or stimulation. With the isotonic magnesium sulphate solution in the bowel the preceding experiments were in part repeated. The urea in the ultrafiltrates of the bowel content and of the blood were now identical, 12.7 mgm. per cent in the former and 12.5 mgm. per cent in the latter. The actual static equilibrium was evidently reached in this type of experiment.

Wells¹ in some unpublished work which preceded ours, and concerning which we were familiar, found that dextrose passes as rapidly into the bowel as into the blood stream if the concentrations be reversed on the two sides, indicating that the mucosa acts toward glucose as a simple semi-permeable membrane.

SUMMARY

1. Urea passes from the blood into the fluid within the lumen of the bowel of dogs and vice versa, readily and in appreciable amounts.

2. With normal saline solution in the living bowel the urea nitrogen level at equilibrium has been found to be somewhat higher on the bowel side than in the blood stream. There was always rapid absorption of fluid from the bowel in these experiments.

3. It made little difference in the equilibrium finally arrived at whether one started with a high concentration of urea in the normal saline solution in the bowel or with the higher level in the blood stream.

4. When the fluid volume in the intestine was kept constant by use of an isotonic magnesium sulphate solution, then at the point of equilibrium, the bowel and the blood stream had an essentially identical urea content.

The writers wish to acknowledge the advice and assistance of Professors M.B. Visscher and A. J. Carlson during the course of this work.

¹ Wells, Herbert S., Department of Pharmacology, Vanderbilt University, Nashville, Tenn., by personal communication.

A NOTE ON THE RELATIONSHIP OF CEREBROSPINAL AND INTRALABYRINTHINE PRESSURES

WALTER HUGHSON

*From the Otological Research Laboratory and the Surgical Hunterian Laboratory,
Johns Hopkins University School of Medicine*

Received for publication May 5, 1932

The anatomic connection between the cochlea and the subarachnoid space is a matter of common knowledge. In clinical medicine this cochlear aqueduct is one of the routes by which infection spreads from the inner ear to the meninges and results frequently in a fatal meningitis. The physiology of the relationship existing between cerebrospinal and intralabyrinthine pressures has, however, never been studied. Weed and McKibben (1919) and Weed and Hughson (1921a, b, c) investigated the relationship between cerebrospinal fluid pressure and intracranial arterial and venous pressures. In their studies they made available for the first time a method of changing cerebrospinal fluid pressure beyond normal physiological limits. This was accomplished by intravenous injections of distilled water which greatly increased intracranial pressure and also by the injection of a strongly hypertonic sodium chloride solution (30 per cent) which reduced cerebrospinal fluid pressure far below its normal limits, in some cases well below zero. At the same time careful studies of arterial and intracranial venous pressures were made and it was definitely shown that the changes in cerebrospinal fluid pressure were not dependent upon circulatory changes. The relationship, therefore, between these abnormal changes in cerebrospinal fluid pressures and circulatory pressures has been definitely established and needs no further confirmation nor discussion here. All of these observations on cerebrospinal fluid pressure were carried out on the cat.

Szasz (1922, 1925, 1926) carried out an important and extensive series of experiments on the relationship between cerebrospinal and intralabyrinthine pressures. Increase of cerebrospinal fluid pressure was produced in every instance by the use of drugs affecting arterial pressure. Reduction in cerebrospinal fluid pressure was produced by placing a cannula in the cisterna magna and allowing fluid to escape. The cochlea manometer—dogs were used exclusively—was introduced through the round window membrane. Each one of these procedures is open to criticism. No drug can change cerebrospinal fluid pressure without affecting

intracranial arterial and venous pressure which alone confirms the result of the present experiment. Repeated dissection and microscopic examination precludes the possibility of introducing a manometer needle 1.5 mm., according to this observer, without damaging some intracochlear structure. Szasz mentions the fact that the presence of blood in the capillary tube indicated damage. A much more accurate method of control is necessary and is fortunately now available. Lorenz (1932) has also investigated the effect of pressure in the labyrinth but from a standpoint which does not bear on the present discussion. He does, however, say that "for good hearing there must be an intact secondary tympanic membrane," an observation amply borne out in the present investigation.

Although not directly apropos to the subject under discussion a method for the study of the physiology of the ear became available approximately a year and a half ago. This method is the Wever and Bray (1930) phenomenon whereby the functional ability of the auditory apparatus can be measured. This method involves the placing of an electrode on the auditory nerve of an experimental animal and the circuit completed by a ground electrode placed in the muscles of the neck. These electrodes are led to an amplifying apparatus which in turn is connected with a telephone receiver or loud speaking apparatus and as sounds are introduced into the cat's ear they are reproduced by the receiving apparatus with absolute fidelity. Without further discussion of this method, therefore, it can be said that a method is available by which the functional ability of the ear can be absolutely controlled. Thanks to this method an accurate study of the physiology of the integral units of the ear is now at the disposal of the investigator. The effect of physiologic changes may be determined from a purely objective standpoint rather than subjective hypothesis. In all the experiments described below the Wever and Bray phenomenon has been used as the method of control.

In the course of a general investigation of the physiology of the ear a study of the effect of reduction of intralabyrinthine pressure by opening the round window membrane and permitting the escape of intralabyrinthine fluid was made and at the same time the effect produced by increasing pressure against the round window membrane was investigated. In a case of the former procedure, where it was reasonable to assume that a reduction of intralabyrinthine pressure resulted, it was found that the functional ability of the ear was decreased for the transmission of high tones. In the latter case, although it was not thought that the effect obtained was due to increased intralabyrinthine pressure (Hughson and Crowe, 1931; Crowe, Hughson and Witting, 1931; Crowe and Hughson, 1931; Hughson and Crowe, 1932) however, it immediately became apparent that careful study of the effect on sound transmission of changes in the intralabyrinthine pressure should be made. In the course of careful

and repeated experimental procedures it was shown beyond all question of doubt that no instrument could be introduced through the round window membrane and fixed sufficiently firmly in place to permit accurate pressure readings without damaging seriously intracochlear structures. With the method described above available any such procedure invariably resulted in damage to the basal osseous spiral lamina and a prompt loss in the ability of the ear to transmit high tones or if severe hemorrhage resulted, in the complete loss of transmission of all tones. Furthermore, within normal physiologic limits there was no method available for changing beyond these limits intralabyrinthine pressure unless it could be demonstrated that marked changes in cerebrospinal fluid pressure might eventually result in corresponding changes of pressure within the cochlea itself without at the same time affecting general systemic arterial and venous pressures except by the use of hypotonic and hypertonic solutions given intravenously.

Thirty experiments have been carried out on cats in the course of the past few months to determine the relationship of fluid pressures in these two body spaces. As stated above it was known that the release of fluid in the cochlea resulting from opening the round window membrane, caused a reduction in intensity of transmission of high tones by the ear. Seven experiments were carried out in which intravenous hypertonic sodium chloride solution (30 per cent) was administered and the effect upon transmission of tones through the ear measured. In every instance the result corresponded to that produced by opening the round window membrane and permitting the escape of intralabyrinthine fluid except that the effect was more striking and more marked. In two experiments hypotonic solution was given intravenously and the effect of these injections also noted. No change could be observed in the ability of the ear to transmit tones except perhaps an increased efficiency at the period of time after the injection when it was known that cerebrospinal fluid pressure had probably reached its highest level. In these early experiments careful study was made of the round window membrane after the injection of the fluid. No particular effect could be observed after the hypertonic solution had been injected because of the proximity of the basal osseous spiral lamina and the normal concave position of the secondary tympanic membrane. After injection of distilled water, however, the membrane could definitely be seen to bulge indicating an increase in intralabyrinthine pressure.

Following these observations transmission tests were made after the injection of both distilled water and hypertonic salt solution taking at the same time cerebrospinal fluid pressure readings made by means of a manometer introduced into the cisterna magna. Following the intravenous injection of distilled water or hypertonic salt solution either a sharp rise or fall of cerebrospinal fluid resulted and if the former, no change in intensity

was noted and if the latter, a marked decrease in intensity of high tones always resulted. Provided the fall in cerebrospinal fluid pressure was great enough all tones from 250 to 4000 d.v. were invariably affected (table 1). In this experiment the values of the various tone frequencies are seen to decline rapidly following the injection of 30 per cent sodium chloride solution. The transmission test made two hours after the end of the injection shows a complete loss of the high tones and a marked falling off of low tone values. No intralabyrinthine pressure readings were made in this particular experiment.

In the cat the anatomic connection between the subarachnoid space and the cochlea is relatively large. A photomicrograph of the cochlear aqueduct is shown in figure 1, its general caliber being apparent in comparison

TABLE 1

Effect of intravenous injection of 10 cc. 30 per cent NaCl solution. Experiment 175

No intralabyrinthine pressures were taken. Note the decline of intensity of all tones as C.S.F. pressure falls.

PROCEDURE	TIME	C.S.F. PRESSURE	FREQUENCY				
			250	500	1000	2000	4000
Transmission test.....	0 min.		30	50	50	42	63
Cistern puncture.....	10 min.	120					
Transmission tests.....	17 min.	95	30	50	47	47	73
	30 min.	95	29	46	49	47	70
Injection begun.....	37 min.	85					
Injection ended.....	47 min.	85					
	52 min.	56	31	47	50	90	
Transmission tests.....	1 hr. 10 min.	0	31	51	61		
	1 hr. 30 min.	-15	39	53	60		
	2 hr. 45 min.	-20	63	62	60		

to the size of the blood corpuscles which appear in its lumen. The arachnoid membrane extends almost completely through the length of the aqueduct and the loosely woven mesh work of the membrane is readily apparent. Attention is called to this fact as it will be referred to in the possible explanation of a phenomenon to be described later.

The necessity of obtaining simultaneous cerebrospinal fluid pressure and intralabyrinthine pressure readings with a study of the effect upon the two produced by intravenous injections of distilled water or a hypertonic sodium chloride solution is obvious. Careful studies were therefore made to establish some method whereby intralabyrinthine pressure changes might be determined. The standard fixed upon was that whatever technical procedure might be employed it should not, of itself, affect the ability of the ear in the experimental animal to transmit all tones in

a perfectly normal manner. Such a standard involved the introduction of some type of recording apparatus into the cochlea without damaging in any way any one of the intracochlear structures. Careful and repeated dissections of the cat's cochlea finally established that there was available one possibility and one alone for the proper estimation of intralabyrinthine pressure. Slightly to the medial side, about 1 mm. anterior from the attachment of the secondary tympanic membrane to the round window niche there is a space which permits the introduction of a needle of fine bore into the scala tympani. Through the kindness of Dr. Isidor Ravdin and Dr. Rubin Lewis of the University of Pennsylvania, a Lewis water bubble manometer was obtained. This instrument is extremely sensitive, is flexible and suited ideally to such pressure estimations. It has the further advantage that it is at all times a closed system and in so small a



Fig. 1. Cochlea aqueduct, showing loosely woven meshwork of arachnoid membrane. In this particular section there is considerable blood in the aqueduct.

space as the cochlea obviously any open system of pressure measurement such as the open capillary manometer used by Szasz, would be far from satisfactory. Figure 2 represents the method used in introducing the needle of the manometer into the cochlea. At first a small hole is drilled in the cochlea wall using a 1 mm. dental drill burr. This hole is drilled under a binocular microscope. With sufficient experience the bone can be drilled to a point where only the thinnest shell of bone is left, figure 2 A. When the experiment is finally set up the cochlear manometer needle is forced through this final thin shell of bone, the joint made tight with ordinary bone wax and pressure readings thereafter may be recorded with the greatest accuracy. Figure 2 B shows a dissection of the medial cochlea wall, the needle in place through the drill hole and emphasizes the fact that it comes in contact with none of the cochlear structures. The

cochlear aqueduct lies almost immediately below the open end of the needle. The procedure employed in setting up the experiment is as follows:

An intratracheal tube is inserted and the animal kept under constant ether anesthesia supplied by compressed air passing over a layer of ether in a Woulfe bottle. The left mastoid bulla is then opened through the neck and the trephine prepared as described above, in the cochlea. The animal is then placed upon its abdomen, small trephines made over both lateral lobes of the cerebellum and an electrode passed through each opening so that action currents may be led off from both right and left auditory nerves. The electrodes are fixed firmly in place, the animal put upon its side, the indifferent electrode placed in the muscles of the neck and a test made for transmission of sound through both ears. This test is regarded

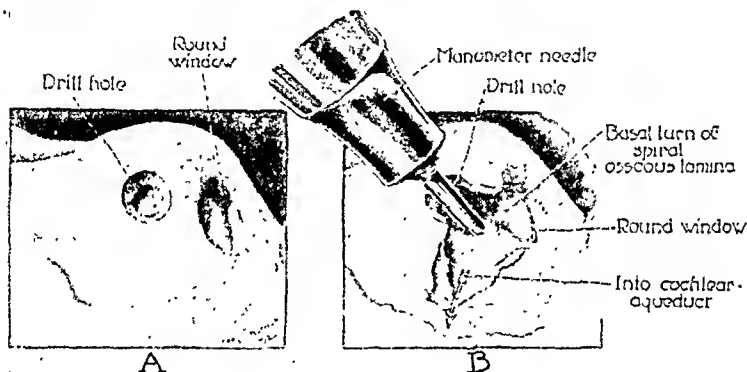


Fig. 2 A. Drill hole in cochlea wall medial and anterior to round window niche. A thin layer of bone is left until manometer needle is introduced.

B. Dissection to show position of manometer needle in cochlea. Point of needle shown entirely free from spiral osseous lamina.

as the normal control. Following this a cistern puncture is made, the needle attached to a water manometer and after an interval of ten minutes, during which time the cerebrospinal fluid pressure usually reaches a fixed level, another sound transmission test is made, in this latter instance of course with a known cerebrospinal fluid pressure. The cochlear manometer needle is then introduced through the trephine opening, held firmly in place with a clamp and another transmission test immediately made to determine whether or not any damage has been done to the cochlear structures. If there is any striking change observed in this particular test on the side on which the cochlear needle has been introduced the experiment is abandoned and ultimate examination under the microscope invariably shows that the basal osseous spiral lamina has been damaged and that hemorrhage has occurred. If the introduction of the cochlear needle is

is included. For the sake of brevity only figures pertaining to the operated ear are given. In every instance the transmission effect on the unoperated ear was in the same direction as in the operated ear. In these tabulations it will be seen that the frequencies 250, 500, 1000, 2000, 4000 d.v. have been arbitrarily chosen. Under these frequencies values in decibels of attenuation are given. These represent the number of decibels necessary to bring into balance intensity of transmission through the cat's ear and the control intensity of the dynamic microphone system. Increase in the number of decibels represents diminished intensity of transmission and decrease in the number of decibels of attenuation means, of course, im-

TABLE 2

Effect of intravenous injection of 50 cc. distilled water. Experiment 169

Intensity values were unaffected by cochlear puncture and for high tones show improvement following increased pressure in labyrinth. Note change of pressures in same direction.

PROCEDURE	TIME	C.S.F. PRESSURE	INTRA- LABYRIN- THINE PRESSURE	FREQUENCY				
				250	500	1000	2000	4000
Transmission test.....	0 min.			34	37	40	86	79
Cistern puncture.....	5 min.	133						
Cochlear puncture.....	10 min.		56.6					
Transmission tests.....	12 min.			30	33	44	80	80
	22 min.	147	57.1					
Injection begun.....	24 min.	157	58.2					
	29 min.	209	71.1					
Injection ended.....	44 min.	236	69.0					
Transmission tests.....	45 min.			30	41	40	50	60
	55 min.	250	68.1					
	1 hr	260	72.7	29	34	38	46	55
Transmission tests.....	1 hr. 10 min.	262	67.2	30	38	40	41	50
	2 hr. 45 min.	262		28	39	40	37	46
	3 hr.	256						

provement in transmission. A detailed description of the amplifying apparatus used and the method involved for measuring changes of intensity produced by different experimental procedures is now in print (Witting, 1932). Up to the present time no effort has been made to make a quantitative estimation of intralabyrinthine pressure changes. The Lewis manometer is approximately thirty times more sensitive than the water manometer used in recording cerebrospinal fluid pressure changes. This factor, however, has not been investigated closely and under the tabulations of intralabyrinthine pressure the direction of the change is always the same as cerebrospinal fluid pressure and for the purpose of this experiment is considered only from a qualitative standpoint.

In table 2 the results of an experiment in which 50 cc. distilled water were administered intravenously are recorded. The animal was prepared as described above, the left ear used and the tabulations recorded represent as shown, procedure, time interval, cerebrospinal fluid pressure, intralabyrinthine pressure and the transmission test made at definite intervals. It can be seen that following the introduction of the cistern needle and the manometer needle for intralabyrinthine pressure no appreciable effect was produced upon normal transmission. Following injection of the distilled water cerebrospinal fluid pressure rose gradually to approximately twice

TABLE 3

Effect of intravenous injection of 50 cc. distilled water. Experiment 171

Illustrates lag in response of intralabyrinthine pressure where maximum was not reached until C.S.F. pressures had started to decline.

PROCEDURE	TIME	C.S.F. PRESSURE	INTRA- LABYRIN- THINE PRESSURE	FREQUENCY				
				250	500	1000	2000	4000
Transmission test.....	0 min.			27	31	40	39	56
Cistern puncture.....	5 min.	167						
Transmission test.....	7 min.			28	35	43	37	52
Cochlear puncture.....	15 min.	180	65.5					
Transmission test.....	17 min.			28	37	39	40	55
Injection begun.....	22 min.	180	67					
	27 min.	216	69.3					
	32 min.	235	70.5					
	35 min.	252	71.4					
Injection ended.....	42 min.	285	74.2					
	43 min.			28	47	44	70	60
	1 hr.	288	81	29	40	43	83	60
Transmission tests.....	1 hr. 5 min.	290	79.5					
	1 hr. 15 min.	258	89					
	2 hr. 45 min.	194	*	27	37	44	46	55
	3 hr. 15 min.	169		26	37	46	42	60

* Pressure in manometer rose so high that bubble was forced into bulb at end of manometer, consequently no further manometer readings could be taken.

its normal height. During this period there was a constant increase in intralabyrinthine pressure and with slight variation a general tendency toward improvement in sound transmission through the ear. The most striking fact and the one of particular interest in this discussion is that the intralabyrinthine pressure changed in the same direction and with slight variations uninterruptedly as the cerebrospinal fluid pressure increased. A final reading of the intralabyrinthine pressure in this experiment was impossible as the needle had become plugged. In table 3 an even more striking effect is apparent for all the factors involved and in addition another effect became apparent which has since been confirmed repeatedly.

It can be seen that with the increase of cerebrospinal fluid pressure there is a constant increase in intralabyrinthine pressure. However, in this experiment it was possible to make readings of the intralabyrinthine pressure over a period of two hours following the intravenous injection of distilled water and there is found to be a certain lag between the rapidity of change in the cerebrospinal fluid pressure and the intralabyrinthine pressure. In other words, after the cerebrospinal fluid pressure had reached its height and had begun to recede somewhat, intralabyrinthine pressure continued to rise. The explanation of this phenomenon seems more or less obvious. The aqueduct, although comparatively large in the cat, is still a minute structure and the interchange of fluid pressures must, of

TABLE 4

Effect of intravenous injection of 9 cc. 30 per cent NaCl solution. Experiment 176

Decline of intralabyrinthine pressure following that of C.S.F. pressure again showing lag in reaching final low level. Note marked loss of intensity of all tones with complete disappearance of two high tones following injection.

PROCEDURE	TIME	C.S.F. PRESSURE	INTRA- LABRYN- THINE PRESSURE	FREQUENCY				
				250	500	1000	2000	4000
Transmission test.....	0 min.			30	40	40	60	63
Cistern puncture.....	10 min.	118						
Transmission test.....	17 min.	118		33	45	43	54	55
Cochlear puncture.....	30 min.		88					
Transmission test.....	33 min.	132	88	28	35	35	67	80
Injection begun.....	36 min.	130	88					
Injection ended.....	47 min.	130	88					
	50 min.	50	87	63	64	63		
	1 hr.	0	87	66	66	70		
Transmission tests.....	1 hr. 15 min.	-23	82	66	63	70		
	1 hr. 30 min.	-26	77	61	68			
	2 hr. 30 min.	5	64.3	61	68	80		
	3 hr.	20	63.2	58	53	70		

necessity, take place very slowly. In short, it is necessary for the cerebrospinal fluid pressure to reach a high level and be sustained at that point for some time before its full effect can be produced upon the intralabyrinthine space. It is also of interest in this experiment to note that this greatly increased pressure had no particular effect upon the transmission of tones through the ear, if any effect was noted, it was in the general direction of improvement of transmission. In table 4 both cerebrospinal fluid and intralabyrinthine pressures are given and the change occurring in the same direction in both pressures following injection of the hypertonic salt solution is apparent. Here again the intralabyrinthine pressure did not reach its lowest point until after the cerebrospinal fluid had itself begun to

return to its normal level. This lag in effect on the intralabyrinthine pressure corresponds exactly to that described in table 3 following the injection of distilled water. The effect of the reduction in the pressure upon transmission is also shown with a prompt loss of high tones and gradual decrease in intensity of all tones.

It is felt that the experiments described above prove conclusively the intimate relationship between cerebrospinal fluid pressure and intralabyrinthine pressure. The dependence of the latter upon the former is perfectly evident. Although the changes described in cerebrospinal fluid pressure as the result of intravenous injections of hypotonic and hypertonic solutions are obviously beyond normal physiological limits, nevertheless it would be impossible by any other method to demonstrate satisfactorily the free communication between these two cavities. The change produced upon the ability of the ear to transmit tones is equally striking though need not be gone into in great detail in the present discussion. It must be emphasized again that the present investigation has to do solely with qualitative changes in intralabyrinthine pressure. The quantitative estimation of these pressures will appear later. Furthermore, it is felt that a method has been developed whereby intralabyrinthine pressures may be obtained without damaging intracochlear structures and in which the accuracy of the pressure readings can be accepted without qualification. It is felt that no other method of determining intralabyrinthine pressure, used up to the present time can be regarded as reliable. This statement is based entirely on the fact that the introduction of the cochlear needle and the effect of its introduction can at all times be controlled by sound transmission tests, a method heretofore unavailable.

CONCLUSION

A new method for determining intralabyrinthine pressure is herewith described and the intimate relationship between cerebrospinal fluid pressure and intralabyrinthine pressure shown. When cerebrospinal fluid pressure is changed by intravenous injection of hypotonic or hypertonic solutions the rise or fall in pressure occasioned thereby is followed directly by intralabyrinthine pressure. There is a definite lag in the change produced in intralabyrinthine pressure, the extremes here not being reached until some time after the limit of change of the cerebrospinal fluid pressure has occurred.

The accuracy of this method is made practically absolute by the simultaneous use of the Wever and Bray phenomenon.

BIBLIOGRAPHY

- CROWE, S. J., W. HUGHSON, AND E. G. WITTING. 1931. *Arch. Otolaryngol.*, xiv, 575.
CROWE, S. J. AND W. HUGHSON. 1931. *Zeitschr. f. Hals-Nasen- u. Ohrenh.*, xxx, 65.

- HUGHSON, W. AND S. J. CROWE. 1931. Journ. Amer. Med. Assoc., lxxxvi, 2027.
1932. Ann. Otol., Rhinol. and Laryngol. (in print).
- LORENZE, H. 1932. Acta, Oto.-Laryngol., xvii, 89.
- SZASZ, T. 1922. Zeitschr. f. Hals- Nasen- u. Ohrenh., iii, 229.
1925. Zeitschr. f. Hals- Nasen- u. Ohrenh., xii, 431. .
1926. Zeitschr. f. Hals- Nasen- u. Ohrenh., xiv, 237.
- WEED, L. H. AND P. S. MCKIBBEN. 1919. This Journal, xxxviii, 512.
- WEED, L. H. AND W. HUGHSON. 1921a. This Journal, lviii, 53.
1921b. This Journal, lviii, 85.
1921c. This Journal, lviii, 101.
- WEYER, E. G. AND C. W. BRAY. 1930. Journ. Exper. Psychol., xiii, 5.
- WITTING, E. G. 1932. Laryngoscope (in print).

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 101

AUGUST 1, 1932

No. 3

SOME CRITERIA OF ACCURACY FOR THE MEASUREMENT OF THE OSMOTIC PRESSURE OF COLLOIDS IN BIOLOGICAL FLUIDS

HERBERT S. WELLS

From the Department of Physiology, Vanderbilt University School of Medicine

Received for publication May 7, 1932

The study of the osmotic pressure of the colloids of biological fluids has, in recent years, cast much light on the mechanisms which are involved in the maintenance of the water balance of the body. The need for accurate measurements of the osmotic pressure of the various body fluids has been recognized in theory, but in practice the determinations have been carried out by such a variety of methods that it is not surprising to find considerable discrepancies in the pressure values obtained by different investigators from measurements on similar fluids. For example, two investigators (1), (2), employing identical membranes, have published widely different average values for the specific osmotic pressure (pressure per gram per cent protein) of the colloids of human blood plasma. The confusion which has arisen as the result of such discrepancies could be resolved by assuming that the osmotic pressure of colloids of any given biological fluid is a quantity so indefinite, so susceptible to slight variations in the conditions under which the determination is carried out, as to provide practically no information of value for the quantitative study of the processes of osmosis as they occur in the animal body. On the other hand, the experience in this laboratory would seem to indicate that the confusion lies not in the variability of the osmotic pressure itself but in the difficulty of controlling the experimental conditions in such a way that a true osmotic equilibrium may be observed.

It is the purpose of this communication to suggest certain criteria of an osmotic equilibrium and to present details of the methods of procedure which have been devised to meet the demands of these specific standards of accuracy, as they have been developed in the course of investigations to be described in subsequent reports. It is hoped that our experience may encourage others to undertake measurements of the osmotic pressure, for,

when proper precautions are observed, it is not a difficult matter to obtain consistent and apparently entirely reliable results.

Criteria of accuracy in the measurement of the osmotic pressure. The properties of the membrane. 1. The membrane shall be sufficiently permeable, sufficiently thin, and of sufficient area in relation to the volume of the sample to allow an equilibrium to be established as quickly as possible between the diffusible constituents of the inner and outer solutions of the osmometer. When the membrane is too "tight" the temporary pressure due to the osmotic activity of these diffusible constituents may be so high and may persist so long that osmotic equilibrium will never become established; for after 24 hours it is often found that spontaneous changes will have occurred in the colloidal state of the solution. Such changes, once started, will usually continue without interruption and in such a manner that the osmotic pressure will fall continuously to zero.

In the study of biological fluids it becomes necessary to demonstrate that the permeability of the membrane remains essentially unaltered by contact with these fluids. Mucus and fats are found, for example, to decrease the permeability of collodion membranes to such an extent that the time for the attainment of osmotic equilibrium becomes definitely prolonged. If the equilibrium is not attained within 24 hours or less the measurement is apt to be of little value, for the reasons given above.

2. While the membrane must be "rapid" it must nevertheless be strictly impermeable to the colloid under investigation.

a. Membranes, if made from collodion, must be water-clear rather than opaque or even slightly "milky" in appearance, for the latter condition is *prima facie* evidence of irregularities in permeability.

b. The specific permeability of each membrane should be determined and its value should lie within the range known, as the result of preliminary experiments, to be suitable for the particular biological fluid under investigation. The range of specific permeabilities of the membranes used should be reported in connection with all published values for the osmotic pressure of colloids.

c. Ultrafiltrates prepared with such membranes should show no trace of the colloid whose pressure is to be measured.

d. After each determination the outer solution should be examined by applying a sensitive qualitative test for the colloid in question, in order that the possibility of leakage of the solution through the membrane or through the junction of membrane and osmometer may be ruled out. The sensitivity of the test should be determined and the figure representing the greatest dilution of the colloid for which the test is sensitive should be published.

e. All determinations of the osmotic pressure should be discarded in which there is the slightest evidence of the presence of the colloid in the outer solution.

f. No osmometer should be used which does not readily allow the application of such a test to the outer solution.

3. The membranes should be free from gross contamination with bacteria or molds.

a. It is preferable to use a freshly prepared membrane for each determination.

b. When membranes must be preserved before they are to be used it is essential to employ an antiseptic which will not alter the permeability.

c. Antiseptics are apt to cause precipitation or other changes in the colloidal state of the sample. Since this factor is difficult to control, it is advisable to wash the membrane free of all antiseptic material before it is used.

The properties of the colloidal solution. 1. There should be no evidence of changes in the colloidal state of the sample during the determination.

a. There should be no visible evidence of precipitation of the material or of adsorption onto the surface of the membrane.

b. Bacteriological or other tests for invisible changes may be carried out as desired, but it is our experience that, if the determination can be completed within 12 to 24 hours, there is little likelihood of such changes causing any appreciable error. As emphasized above, it is the spontaneous change which may occur in a bacteriologically sterile medium which produces the greatest errors.

c. Spontaneous changes in the colloidal state, often (but not always) accompanied by a visible precipitation of the material, will occur much more quickly at a temperature of 0°C . than at higher temperatures (15°C . to 38°C .). It would therefore seem desirable to carry out the determinations at room temperature, for the spontaneous changes of lower temperatures and the more rapid bacterial changes of higher temperatures are thus avoided.

The properties of the outer solution. 1. Theoretically an ultrafiltrate prepared from the colloidal solution in question would allow the most rapid and most accurate determination of the osmotic pressure. In practice it is found that the time for the attainment of equilibrium is not reduced by the use of ultrafiltrates prepared in the usual manner. Until this question has been further investigated we may assume that in the actual process of filtration, as ordinarily carried out, certain of the relatively non-diffusible constituents are concentrated in the solution and that the filtrate is not a true ultrafiltrate.

For mammalian fluids it has been found that 0.9 per cent NaCl solution is entirely satisfactory, and that with its use the same equilibrium is reached in the same time as with "ultrafiltrate" or Locke solution.

2. The volume of the outer solution should be as small as possible in relation to the volume of the inner solution, in order that the changes in

composition of the latter, resulting from the outward diffusion of certain of its constituents, may be as slight as possible.

Criteria of osmotic equilibrium. 1. In order to be certain that a true osmotic equilibrium has been attained the volume of the solution, as judged by the height of the meniscus in the capillary tube of the osmometer, must be observed to remain absolutely constant, at constant pressure and constant temperature, for a considerable length of time. Depending on the permeability of the membrane and other factors the observation may have to be continued for minutes, hours, days, weeks or even for several months in order to be certain that one has to deal with a true equilibrium. The occurrence of any detectable change of volume during this time should be grounds for discarding the determination. Unfortunately, to insist on this simple but essential criterion of an osmotic equilibrium is to insist that the majority of the figures which have appeared in the literature are unreliable.

a. The temperature of the osmometer should remain constant within a few hundredths of a degree in order that the volume of the contents may remain constant; otherwise with changes in temperature irregular movements of the meniscus will occur which are misleading.

b. Volume changes due to osmosis or ultrafiltration should be reduced to the absolute minimum, for the changes in concentration of the colloid produced at the surface of the membrane by the movement of fluid through the membrane have been found to give rise to changes in the osmotic pressure which may be considerable and which may require many hours for readjustment. For this reason it is desirable that an accurate balance of pressures be maintained by frequent adjustment of the external pressure which is applied to the solution by means of a water manometer.

c. The osmometer tube should have a bore which is sufficiently narrow to favor ease of observation of movements of the meniscus. However, the smaller the bore, the greater is the correction for the capillary rise of fluid in the tube. Consequently the error of the determination of the capillarity should be taken into account in relation to the magnitude of the osmotic pressure to be measured. For the measurement of pressures above 100 mm. water a narrow bore (0.5 mm. or less) is permissible; while for the estimation of lower pressures the tube should be relatively wide (1 to 2 mm.).

d. For the particular osmometer and for the standard membranes used in this laboratory, osmotic equilibrium should be considered as having become established only when the meniscus of the fluid in the osmometer tube has been observed to remain steadily in one position, within a few hundredths of a millimeter for at least 2 hours. At the end of this time it must be demonstrated that a change of 0.5 to 2.0 mm. of water of the pressure applied will, within 5 minutes, cause the meniscus to move for a

measurable distance and at a steady rate, either up or down, according to the direction of pressure change.

e. Determinations of the osmotic pressure should be carried out in duplicate whenever sufficient material is available, and the values obtained in the duplicate determinations should not differ by more than the greatest error of determining the correction for capillarity. This error should rarely exceed 10 per cent of the total capillary correction.

DETAILS OF THE METHOD USED IN THIS LABORATORY. *The osmometer.* The micro-osmometer of Krogh (3), (4) possesses the unique advantage of requiring relatively small amounts of biological fluids for the accurate determination of the osmotic pressure of colloids. A sample of from 0.2 cc. to 0.7 cc. is sufficient, and the capacity of the osmometer may be adjusted in each case to the volume of the sample available, within the limits just given. The osmometer possesses the theoretical advantage of allowing the outer solution, the volume of which is not greater than 0.1 cc., to come into equilibrium, so far as diffusible substances are concerned, with the sample, a result which may be accomplished with minimal changes in the composition of the latter. Furthermore, by this method an osmotic equilibrium is actually attained. The so-called dynamic methods, so much in use at the present time, will, unless they are employed under almost ideal conditions, often lead to gross errors. Finally, with the Krogh osmometer it is possible to test the outer solution for the presence or absence of proteins as a check on the strict semi-permeability of the membranes with respect to the substance whose osmotic pressure is being measured.

The one disadvantage of the method, as originally described, has been the difficulty of preparing suitable membranes. This difficulty has been largely overcome in this laboratory during the course of experiments in which it was desired to measure the osmotic pressure of blood serum and lymph of dogs.

Preparation of membranes. The collodion solution is prepared by dissolving 8 grams of dry "Parlodion" shreds for each 100 cc. of solution in equal volumes of absolute ethyl alcohol and anhydrous ether. As it requires several days for the collodion to become completely dissolved it is advisable to prepare a stock solution of 500 cc. or more. The solution should be prepared and preserved in a glass stoppered vessel.

In the original method the sacs are formed on glass capillary tubes of approximately 4 mm. outside diameter, and having rounded tips. These tubes were filled with mercury to facilitate removal of the membranes. I have considered it desirable to omit the use of mercury, due to the fact that droplets of this material almost invariably become enclosed in the collodion at the tip of the sac. Oxidation of this mercury in the presence of ether leads to the formation of a reagent which may precipitate serum

proteins. With a stopcock sealed to the upper end of the glass tube, air may be trapped in the bore in such a way that, on dipping the tip of the tube into collodion two or three times, with intervals for drying of 15 minutes or longer, a hard cap may be formed which will prevent the flow of collodion into the bore of the capillary during the formation of the membrane.

Whenever it is desired to make up a batch of membranes, a 50 cc. glass-stoppered cylinder is filled to the mark with fresh collodion from the stock solution. One of the glass capillary tubes is dipped into the solution in the cylinder to a depth of about 8 cm. It is then raised until only the tip remains in contact with the surface of the solution, and the excess collodion is allowed to drain for exactly 90 seconds. The atmosphere of alcohol and ether vapors present in the upper part of the cylinder will prevent drying, thus facilitating a free and uniform drainage. The tube is then removed and allowed to dry in a vertical position, at room temperature, for 60 seconds. It is then dipped a second time and allowed to drain for 120 seconds, whereupon it is immediately placed, still in the vertical position, in a drying chamber through which air is constantly flowing. Such a chamber may be made from a 5 pound ether can by cutting in the top a number of holes of a diameter slightly greater than that of the glass tubes. The air is led in through the central spout of the can, and is conducted to the bottom through a glass tube, whence it escapes to diffuse upward, past the membranes, and out through the holes. The air coming from the pressure supply is passed through concentrated sulphuric acid to remove water vapor, through soda-lime to remove acid spray, through a filter of cotton gauze to remove lime dust, and finally through ethylene glycol. The trace of glycol present in the air keeps the membranes pliable enough so that they can be removed from the glass tubes after all the alcohol and ether has been allowed to evaporate. For complete drying approximately 24 hours are required.

Some care is required in the removal of the dry sacs from the glass tubes. The sac is first cut through at a distance of approximately 4 cm. from the tip. The stopcock is opened to allow the membrane to fill with air while it is being removed. The tip is then loosened by applying a heavy, twisting pressure with the thumb and forefinger. The membrane should then be slowly pushed off, pressure being applied just back of the tip. In order to prevent the membranes from becoming soiled or greasy it is a good idea to cover the fingers with several layers of gauze. Should it prove to be impossible to remove the sac at the first attempt it will be necessary to replace it in the drying chamber for several hours in order that it may become further softened by the deposition of glycol. The sacs so prepared are hard, dry and transparent, presenting none of the milky appearance of the "wet" membranes prepared in the ordinary way. The least trace

of water vapor in the air will cause the "milky" precipitation of collodion as it dries, with the consequence that marked irregularities in permeability will occur.

The membranes are now placed in a tightly stoppered jar containing a solution prepared by adding 5 cc. of water to 95 cc. of absolute ethyl alcohol. It has been found by trial that this concentration of alcohol will, in 24 hours or less, cause the dry membranes to swell to the proper degree of permeability. The discovery of this general method of treating dry membranes to render them permeable is attributed to Brown (5).

After being allowed to swell in alcohol the membranes are rinsed many times, inside and out, in distilled water, and preserved in 0.9 per cent NaCl solution saturated with chloroform. It has been found that the acriflavine used by Krogh as an antiseptic is adsorbed onto the membranes, rendering them brittle and increasing their permeability in an irregular manner. The chloroform does not have this effect, but it possesses the disadvantage that it will precipitate protein from the serum. Consequently it must be removed by thorough rinsing in fresh saline before the membrane is to be used.

Standardization of membranes. The permeability of the membranes used has been repeatedly tested by the determination of the rate of filtration of water. This method of standardization has proved helpful in the hands of Zsigmondy (6), Krogh (4), and others. However, as these authors have not included in their calculation of the filtration rate the factor of the thickness of the membrane, their "filtration numbers" are of little value to anyone else. In order that the membranes used by different investigators may be compared it would be desirable to adopt some definite standards for the measurement of permeability. In the absence of such an accepted standard I have chosen to define as a measure of the "specific permeability" of a membrane, the number representing the cm.^3 of water filtered per second through 1 cm.^2 of membrane of 1 cm. thickness, by a hydrostatic pressure difference of 1 atmosphere (1 atm. = 760 mm. Hg) at a temperature of 20°C.

The filtration rate for water has been determined usually at average pressures of from 200 to 300 mm. of mercury. The sac is connected to a 1 cc. pipette, calibrated in hundredths of a cubic centimeter which replaces the osmometer tube of the usual set-up. Pressure is applied by air transmission from a mercury manometer. The time required for the filtration of a definite volume of water is determined, as is the mean pressure during this time. In calculating the area of the membrane the tip, which is relatively dense and thick, is neglected. For the measurement of the average thickness a micrometer gauge is employed which reads to 0.01 mm., and the membrane is measured while wet. The tip of the membrane is cut off and it is slit lengthwise. The thickness of the membrane is

measured at each end and in the middle and a figure is chosen which seems to express as nearly as possible the mean thickness. As this measurement involves the destruction of the membrane it must of course be carried out either after the membrane has been used for a determination of osmotic pressure, or on a sample membrane which is sacrificed for the purpose of obtaining an idea as to the probable permeability of the other membranes of the same series. It very rarely happens that significant variations will occur in any given group of membranes, prepared at the same time and with due regard for uniformity of treatment. Where v is the volume in cubic centimeters filtered in t seconds at a mean pressure of p atmospheres through a membrane whose area is a square centimeters and whose thickness is h centimeters, the specific permeability is $P = \frac{vh}{atp}$. Membranes

prepared as directed should possess permeability numbers lying in the range of 20×10^{-8} to 40×10^{-8} . However, accurate and sufficiently rapid determinations can be carried out on serum or lymph with membranes whose permeabilities vary between 10×10^{-8} and 50×10^{-8} . The membranes should not be thicker than 0.04 to 0.08 mm.

Should it be found by trial that membranes made by this method are either too permeable or not permeable enough it is only necessary to vary slightly the concentration of alcohol used for swelling them. The permeability will be increased by a higher concentration and, conversely, will be decreased by a lower concentration of alcohol.

It has been found that membranes softened in a solution consisting of 98 volumes of alcohol and 2 volumes of water are distinctly permeable to the serum proteins. With one such membrane, whose specific permeability number was 340×10^{-8} , the pressure continued to fall for 20 hours, without a true equilibrium becoming established. On the other hand, membranes softened in a solution consisting of 90 volumes of alcohol and 10 volumes of water are so tight that it may require from 12 hours to several days for the attainment of a true equilibrium. The permeability numbers of these membranes were not determined. When the equilibrium pressure could be measured with these membranes the value was found to be the same as was obtained with the more permeable membranes recommended above. The use of such tight membranes is to be avoided, for the pressure falls so slowly toward the equilibrium that one is very apt to be misled and to record pressure values which are too high. Even with scrupulous care, it may not be possible to obtain the correct equilibrium, for in the time necessary to attain this state the proteins of the serum will frequently undergo spontaneous changes which will cause a continuous falling of the pressure below the original equilibrium value. In fact the pressure may never reach a steady state, but often falls gradually to zero in the course of 4 or 5 days.

The difficulties inherent in the use of tight membranes will obviously appear whenever a membrane, originally of the correct permeability, becomes "plugged" by the adsorption of fat, mucus, or other material contained in certain types of biological fluids. For example, samples of bile which contain considerable quantities of mucus may exhibit pressures up to 1 atmosphere, which will fall off very slowly during the course of 4 or 5 days. Putrefaction or precipitation of the material may occur before the pressure has fallen lower than 0.5 atmosphere. The membranes used may originally be readily permeable to bile salts and even to serum proteins, but after contact with the mucus of bile the specific permeability may be reduced to such an extent that it becomes impossible to determine whether or not bile exhibits a true osmotic pressure of colloids. When the bile is aspirated from the gall bladder of the living animal, however, it often contains relatively little mucus, and under these conditions the osmometer pressure rapidly falls, within 24 hours, to atmospheric, and the specific permeability of the membrane shows relatively little change. The relatively high content of fat in the intestinal lymph of animals fed on cream may decrease the permeability of the membrane in a similar manner so that the time necessary for the attainment of osmotic equilibrium will be considerably prolonged, as compared with the usual time required for serum or for lymph of low fat content.

The time necessary for the attainment of osmotic equilibrium may also be unduly prolonged, with the usual possibilities of error in the measurements, when the membranes employed are too thick. A membrane which possesses the correct specific permeability may nevertheless be impractical if its thickness so retards diffusion between the inner and outer solutions of the osmometer that an equilibrium cannot become established before bacterial or other changes in the proteins have altered the colloidal state of the material.

The determination of the osmotic pressure. The details of the construction and the general method of use of the osmometer will not be described as they have been adequately treated in the article by Krogh and Nakazawa (2). Certain suggestions, coming out of an actual experience with the method may, however, be in order.

A Dewar flask (pint size "thermos" bottle filter) may be used in place of the more expensive and less practical thermostat bath. This flask is filled with water at room temperature. Two osmometers, supported by a suitable metal bracket, may be placed in each flask.

The bore of the osmometer tube may be reduced to less than 1.0 mm., the size recommended by Krogh. This provides for more rapid changes in the level of the meniscus.

Although a cathetometer or other form of telescope provided with a cross-hair is desirable for the observation of the movements of the meniscus,

it becomes necessary to provide one such instrument for each osmometer in use at any one time. The expense of such a series of telescopes may be avoided if a cross-hair is supported directly against the osmometer tube. This may be done by cementing a hair across a metal clip, such as is provided with the glass tuberculin syringes in common use. This clip is provided with a spring which will support it against the osmometer tube. The clip is moved up or down as the level of the meniscus changes. By observation with a pocket lens the direction of movement of the meniscus with respect to the cross-hair may be detected.

The pressure of a water manometer is required, in addition to that of the column of fluid in the osmometer, to balance the osmotic pressure. I have found it desirable to leave the connection to the manometer open from the very beginning of the determination. The pressure is adjusted from time to time in such a way as to prevent any appreciable volume change of the fluid in the osmometer. This precaution is essential, for it has been found that if too great a pressure is applied there will occur, as a result of the ultrafiltration, an increase of the concentration of protein at the surface of the membrane sufficient to raise the osmotic pressure considerably above its equilibrium value. Conversely, if the applied pressure is too low the concentration at the surface will be lowered and too low an osmotic pressure will be registered. In either case it may require from 2 to 12 hours for a complete restoration of the equilibrium conditions. It should be noted, however, that even when the volume of the solution is kept constant the initial pressure necessary to balance the system will be considerably higher than that required at equilibrium. This temporary high pressure is undoubtedly due to the presence in serum of slowly diffusing substances which exert an "osmosis" pressure until they have diffused into the outer solution.

The true osmotic equilibrium should be established within 1 to 6 hours. In some instances the equilibrium pressure is found to remain constant to within 0.5 mm. of water for 4 to 5 days. In most cases the pressure will fall off very gradually, from 5 to 15 millimeters a day. This fall is presumably due to spontaneous changes in the proteins, for the same phenomenon can occur in the absence of bacterial contamination or passage of protein through the membrane. Occasionally a distinct precipitation of protein will appear as the result of bacterial action but this seldom occurs in less than 48 hours. Equilibrium is not considered as having become established until the balancing pressures check within 1 or 2 mm. of water for at least 2 hours. When determinations are carried out in duplicate a difference of more than 5 mm. in the two determinations is exceptional.

After the determination has been completed the outer solution should be tested for proteins. The nitric acid ring-test will readily detect the

presence of serum protein at a dilution of 1:40,000. The result of this test should be negative if the determination is to be considered reliable.

In order to determine the correction for the capillarity of the osmometer tube it is necessary to take the mean of the heights attained by the serum in falling and in rising toward the equilibrium level. This is necessary because, in the case of a capillary of narrow bore, the serum may appear to have come to rest when actually it is still 5 to 10 millimeters from the point of equilibrium.

TABLE 1

SAMPLE	TOTAL PROTEIN	OSMOTIC PRESSURE OF COLLOIDS	MEAN SPECIFIC OSMOTIC PRESSURE	SPECIFIC PERMEABILITY OF MEMBRANES USED	PERIOD OF OBSERVED EQUILIBRIUM, TIME FROM START
	<i>per cent</i>	<i>mm. H₂O</i>	<i>mm. H₂O</i>	<i>cm.² per sec. per atm. $\times 10^{-8}$</i>	<i>hours</i>
Dog serum	4.65	182	40	22	2 to 4
		187		22	2 to 5
Dog serum	5.75	252	44	26	3 to 8
		250		32	4 to 6
Dog serum	6.41	258	40	51	3 to 25
		256		31	2 to 4
Dog serum	6.68	276	41	36	6 to 120
Dog serum	6.79	275	40	37	3 to 20
		271		41	2 to 20
Dog serum	6.86	325	48	22	2 to 5
		330		18	3 to 7
Dog serum	7.12	329	46	21	2 to 4
		330		32	2 to 5
Human serum	7.50	370	49	35	5 to 7
		366		37	3 to 10

Table 1 presents typical examples of the results obtained by the use of the Krogh osmometer. By reference to the last column of the table it will be noted that most of the experiments were terminated at the end of 4 to 8 hours. However, in several instances the osmotic equilibrium was observed to remain absolutely constant for a much longer time. It is believed that the data will justify the choice of a two-hour period of observation of a constant pressure as a sufficiently rigorous test of the attainment of the true osmotic equilibrium.

For further examples of results obtained by the method the reader is referred to the next article in this journal (pp. 421 to 433).

SUMMARY

1. The occurrence of considerable discrepancies in published values for the osmotic pressure of colloids of similar biological fluids indicates the necessity for the adoption of standard criteria of accuracy for such measurements.

2. Definite criteria are suggested.

3. Details of the procedures which have been evolved to meet the demands of these criteria are presented.

a. A method for preparing collodion sacs of the proper degree of permeability for the determination of the colloid osmotic pressure of serum or lymph is described.

b. A standard designation for the "specific permeability" of membranes is proposed and the method of standardization described.

BIBLIOGRAPHY

- (1) GOVAERTS, M. P. 1927. Bull. de l'acad. Roy. de Med. de Belgique, xiii, 356.
- (2) VERNEY, E. 1926. Journ. Physiol., lxi, 319.
- (3) KROGH, A. 1924. The anatomy and physiology of capillaries. New Haven.
- (4) KROGH, A. AND F. NAKAZAWA. 1927. Biochem. Zeitschr., clxxxviii, 16.
- (5) BROWN, W. 1915. Biochem. Journ., ix, 320; 591.
- (6) ZSIGMONDY, R. AND W. BACKMANN. 1918. Zeitschr. f. anorg. u. allgemein. Chem., ciii, 119.

THE CONCENTRATION AND OSMOTIC PRESSURE OF THE PROTEINS IN BLOOD SERUM AND IN LYMPH FROM THE LACTEALS OF DOGS

HERBERT S. WELLS

From the Department of Physiology, Vanderbilt University School of Medicine

Received for publication May 7, 1932

The lymph obtained from the thoracic duct of a resting animal is presumably derived, for the most part, from the liver and intestines (1). In order to exclude the lymph from the liver and thereby to obtain from the thoracic duct a so-called "intestinal" lymph, various workers have employed operative procedures such as ligation of the lymphatics of the liver (1) or removal of this organ (2). Such procedures are difficult to carry out and may not be entirely reliable, for they necessarily involve manipulation of the splanchnic organs to an extent which may well result in the production of marked changes in the composition of the lymph from the intestine. It has been found in this laboratory that a true intestinal lymph can be obtained by direct aspiration from the lacteals of the mesentery, a procedure which was developed in the course of an investigation undertaken primarily for the purpose of determining the relation of the absorbing force of the intestine to the osmotic pressure of the colloids of the intestinal lymph (3). During this investigation the concentration and osmotic pressure of the proteins of this lymph, as well as of blood serum from the same animals, were determined, and the mesenteric venous pressure was measured in the majority of animals. Analysis of these data elicits information that is obviously more pertinent to the question of the normal mechanism of lymph formation in the intestine than is provided by studies carried out on lymph from the thoracic duct. The results of this study of the intestinal lymph indicate that the protein content of the lymph is determined within fairly definite limits by the protein content of the serum; that the osmotic pressure for one per cent of protein of the lymph is of the same magnitude as that of serum; and that the mesenteric venous pressure is sufficiently high, in relation to the calculated "effective" osmotic pressure of the blood, to account for the continuous formation of intestinal lymph by a process of rapid filtration of protein-containing fluid through the capillaries.

METHODS. *Collection of lymph from the lacteals.* Although it is possible to collect lymph from a fasting animal, a good deal of massage of the

gut is necessary in order to obtain a large enough sample. It is probable that the lymph obtained in this way will differ in composition from the normal intestinal lymph, for as the result of handling the gut, vasomotor changes as well as changes in the permeability of the capillaries may be presumed to take place. When the animal is in the digestive state, however, the lacteals of the intestinal wall and of the mesentery are so well filled that it is possible to obtain the desired amount of lymph with a minimum of manipulation of the gut. Consequently in each instance the animal has been fed a large meal of meat, bread and skimmed milk, in order to insure an adequate filling of the lacteals with the digestive lymph. Enough food was given in the evening so that an excess remained in the cage the following morning.

The dogs were anesthetized by the intravenous injection of 0.3 gram of sodium barbital per kilo of body weight. When the abdominal cavity had been opened the lymphatics of the mesentery were usually found to be well filled with a milky lymph.

The lymph was collected by introducing a 24 gauge hypodermic needle into the lymphatic vessels of the mesentery of the jejunum. The lymph was aspirated by gentle suction into a test tube, on the walls of which a thin layer of sodium oxalate had been precipitated by evaporation. Suction was provided by appropriate connections to a filter pump and to a pressure regulator which provided a constant negative pressure of approximately 7 cm. of water. The needle was inserted in turn into several of the larger lymphatic trunks. By rolling the gut lightly between the fingers it was usually possible to obtain 0.1 cc. or more at each puncture. No attempt was made to exhaust the available supply. When the lymph ceased to flow freely the needle was withdrawn and inserted into another lymphatic trunk. Proceeding in this manner it was relatively easy to collect from 0.5 cc. to 1.5 cc. of lymph from the upper 30 to 40 cm. of jejunum. From 0.3 to 0.5 cc. of the sample was sufficient for an osmotic pressure determination. The remainder was used at once for protein analysis. After the osmotic pressure had been determined the lymph was removed from the osmometer sac and used for the remaining chemical analyses.

In all cases the lymph has been milky-white in color and therefore apparently free from contamination with blood.

Measurement of the mesenteric venous pressure. In most instances an interval of from 1 to 3 hours elapsed between the collection of lymph and the measurement of the venous pressure. This interval of time was required for the determination of the absorbing force of the gut, a procedure which required that a loop of jejunum, in the region from which lymph had been collected, be isolated according to the method which has been described elsewhere (3). For the measurement of the venous pressure a

hypodermic needle connected to a manometer containing physiological salt solution was inserted into a side branch of a mesenteric vein of the isolated loop. The needle was directed centrally so as to give readings which represent as nearly as possible the lateral pressure in the nearest connecting vein. The measurement was repeated several times. In a few instances the pressure was also measured before and again immediately after the collection of lymph. The values so obtained were approximately the same as those found after the absorption period.

For the measurement of the venous pressure the same level of reference was chosen as was adopted for the determination of the absorbing force, viz., the plane of the horizontal diameter of the lumen of the isolated loop.

Collection of blood samples. Blood for the determination of the concentration and osmotic pressure of proteins was withdrawn from the heart immediately after the measurement of the venous pressure. The blood was allowed to clot, centrifuged at high speed for 10 minutes, and the serum removed at once.

Determination of the total protein and albumin and globulin fractions of serum and lymph. The protein concentrations of the various fractions were calculated from direct nitrogen determinations by the micro-Kjeldahl aeration method of Folin and Farmer (4). For the titrations, freshly prepared and frequently standardized solutions of 0.01 N NaOH and HCl were used. Blank determinations were carried out on the reagents and the correction found to be negligible. The accuracy of the method was checked by the analysis of various dilutions of a standard urea solution. The greatest error of the method as used appears to be of the order of 0.1 gram of protein per 100 cc. For the total nitrogen determinations 0.1 cc. of serum or lymph was used, for the accurate measurement of which special 0.1 cc. pipettes were employed. Duplicate analyses were carried out in almost every instance.

For the separation of the globulin fraction the method of Howe (5) was employed, using 19 volumes of 22.5 per cent solution of Na_2SO_4 to 1 volume of the sample. The mixture, so prepared, was allowed to stand in the incubator at 38°C for 12 hours in order to insure the complete flocculation of the globulins. The presence of fat in the lymph seemed to favor rather than hinder the separation. In almost every instance a water-clear filtrate was obtained for the analysis of the albumin nitrogen. The globulin was calculated by difference.

The determinations of non-protein nitrogen were carried out on tungstic acid filtrates of serum and lymph, prepared according to the standard method of Folin. Such determinations were not carried out in the seven earlier experiments. The figures for these experiments have therefore been arbitrarily corrected by subtracting from the values for total nitrogen and for albumin nitrogen of lymph and of serum respectively corrections

which are the approximate averages of values for non-protein nitrogen found in the seven later experiments. These corrections are, for lymph 0.3 per cent and for serum 0.22 per cent, calculated as protein. Such a correction of the figures for the protein of serum is completely justified by the fact that the non-protein nitrogen content of serum is a relatively invariable quantity. Thus the seven determinations which were carried out gave values of 0.26, 0.22, 0.22, 0.22, 0.21, 0.22, and 0.23 per cent, as protein. In the case of lymph the values found in seven determinations were 0.15, 0.25, 0.31, 0.28, 0.51, 0.42 and 0.35, with an average of 0.324 per cent, calculated as protein. The variation is greater than was the case for serum. Nevertheless, the error which is introduced by accepting an average value of 0.3 per cent for the non-protein nitrogen of lymph, calculated as protein, is not sufficiently great to invalidate the conclusions which have been drawn from the figures as given.

The determination of the osmotic pressure of colloids of serum and lymph. Details of the method are described elsewhere in THIS JOURNAL (6).

In no experiment was enough lymph collected to allow the determinations to be carried out in duplicate. In all cases a cathetometer was used for observation of the movements of the meniscus in the osmometer tube. By the aid of this instrument movements of less than 0.01 mm. can readily be detected. Only when the meniscus had remained absolutely steady for 2 hours or longer was it considered that a true osmotic equilibrium had been attained. In several instances the equilibrium pressure was found to remain unchanged for as long as 48 hours. During this time a change of from 0.5 mm. to 2.0 mm. of the pressure applied by means of the water manometer was sufficient to cause, within 5 minutes or less, a definite movement of the meniscus. In most instances it was possible to complete the determinations within 5 to 10 hours.

In order to determine whether the sodium oxalate used to prevent clotting of the lymph would affect the osmotic pressure of colloids, a control experiment was carried out. A pint of cream was administered to a dog by stomach tube in the evening. Next morning the animal was anesthetized with barbital, the thoracic duct cannulated and 20 cc. of lymph collected. The lymph was defibrinated by shaking with glass beads. The sample was then divided into three portions. One portion was completely saturated with sodium oxalate. The second portion was placed in one of the tubes used for collection of lymph from the lacteals, on the walls of which a few milligrams of oxalate had been deposited by evaporation from a saturated solution. The third portion remained untreated. The osmotic pressure of the colloids of each sample was then determined. The values found were successively 171 mm., 185 mm. and 181 mm. It appears that complete saturation with sodium oxalate reduced the osmotic pressure approximately 6 per cent, whereas the amount of oxalate ordinarily

added in the collection of lymph caused no significant change in the osmotic pressure of colloids.

For the determination of the osmotic pressure of serum a single osmometer was set up in each of the first 6 experiments, but in the last 8 experiments duplicate determinations were carried out. The greatest variation between the pairs of values so found was 15 mm. of water: it is unusual to

TABLE 1

The protein content and osmotic pressure of colloids of blood serum and of lymph from the lacteals

Data arranged by experiments in ascending order of the total protein content of the sera. Figures marked with an asterisk have been arbitrarily corrected for non-protein nitrogen, as explained in the section on methods.

NUMBER OF EXPERI- MENT	ALBUMIN		GLOBULIN		ALBUMIN GLOBULIN		TOTAL PROTEIN		PROTEIN RATIO LYMPH SERUM	SPECIFIC OSMOTIC PRESSURE		TOTAL OSMOTIC PRESSURE	
	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph		Serum	Lymph	Serum	Lymph
	per cent	per cent	per cent	per cent	ratio	ratio	per cent	per cent		mm. H ₂ O	mm. H ₂ O	mm. H ₂ O	mm. H ₂ O
12	2.69	1.19	1.96	0.98	1.37	1.22	4.65	2.17	0.47	39.8	32.2	185	70
13	2.50	0.83	2.50	0.84	1.00	0.99	5.00	1.67	0.33	41.0	34.1	205	57
14	2.53	1.09	2.61	0.97	0.97	1.12	5.14	2.06	0.40	37.0	29.1	190	60
3	*3.03		2.29		1.32		*5.32	*2.75	0.52	45.7		243	[110]
7	*3.57	*1.97	2.18	1.07	1.64	1.84	*5.75	*3.04	0.53	43.6	45.3	251	152
4	*3.08	*1.68	3.13	1.10	0.98	1.53	*6.21	*2.78	0.45				
8	2.77	2.14	3.64	1.85	0.76	1.16	6.41	3.99	0.62	40.1	39.6	257	158
5	*3.07	*1.56	3.61	1.64	0.85	0.95	*6.68	*3.20	0.48	41.3	40.0	276	128
2	*2.98		3.80		0.78		*6.78			42.6		289	142
9	3.03		3.76		0.81		6.79	3.93	0.58	40.2	40.1	273	170
10	4.02	2.15	2.84	1.07	1.41	2.01	6.86	3.22	0.47	47.8	43.2	328	139
11	4.39	2.85	2.73	1.70	1.61	1.67	7.12	4.55	0.64	46.3	47.1	330	214
6		*1.82		1.52		1.20		*3.34			37.4		125
1												237	89
Aver- age....	3.18	1.72	2.80	1.25	1.17	1.39	5.98	2.97	0.499	42.3	40.8	256	125

observe so great a discrepancy. The least variation was 1 mm. and the average was 4.5 mm.

The collodion sacs used for the determinations of the osmotic pressure of serum and of lymph in the last 10 experiments were standardized according to their rates of water filtration. The specific permeabilities (6) so determined varied from 18×10^{-8} to 51×10^{-8} cm.² per second per atmosphere.

PRESENTATION AND DISCUSSION OF DATA. *Albumin and globulin fractions.* From an examination of the data, which are presented in table 1,

it appears that the albumin content of the lymph is lower than that of the serum in each case. The same relationship holds for the globulin fractions. The albumin:globulin ratio on the other hand is higher for lymph than for serum, in most instances. The finding of relatively more albumin than globulin in the lymph is in accord with the observation of Munk and Rosenstein (7) who found albumin:globulin ratios of 4.0 and 2.5 in two samples of lymph from a fistula in a patient. Morawitz (8) who, so far as I am aware, is the only investigator to have previously determined the albumin and globulin fractions of serum and lymph in the same animal, found from the analysis of two samples of lymph from the thoracic duct of dogs ratios which were greater than unity and somewhat higher, in both cases, than the corresponding ratios for serum. The ratios for lymph were 1.56 and 1.58 and those for serum were 1.25 and 1.44. In general the available data support the generally accepted idea (8), (9), (10) that serum albumin, having a lower molecular weight than serum globulin, can pass more readily through the capillary wall.

Ratios of protein of lymph to protein of serum. Although the total protein content of the lymph is lower, in each case, than that of the serum, it appears that with increasing protein concentration of the serum there is an increase in the corresponding lymph protein content. Corresponding to the lowest value for the protein concentration of lymph, 1.67 per cent, we find a serum protein of 5.0 per cent; and for the case of the highest value of lymph protein content, 4.55 per cent, the protein of the serum is present at a concentration of 7.12 per cent. This relationship is brought out more clearly in the column showing the ratio of protein of lymph to protein of serum. The lowest value of the ratio is 0.33, the highest is 0.64, the mean is 0.50 and the average deviation from the mean is ± 0.072 . Exactly similar ratios appear, by calculation, from the observations of Loewen, Field and Drinker (10) on serum and on the lymph from the thoracic duct of a series of dogs. From the data on eight animals in which there was encountered a range of protein concentrations for blood of 4.19 per cent to 8.84 per cent, and of lymph from 2.12 per cent to 4.93 per cent, calculation gives the following ratios: 0.55, 0.56, 0.51, 0.39, 0.44, 0.64, 0.45 and 0.30, of which the lowest value is 0.30, the highest is 0.64, the mean is 0.48 and the average deviation from the mean is ± 0.085 . In addition, ratios ranging from 0.36 to 0.76, with an approximate average of 0.55 may be found by calculation from the data for normal animals of Meyer-Bisch and Günther (11) who published a large number of figures on the protein content of serum and of lymph from the thoracic duct of dogs. Arnold and Mendel (12) obtained ratios of 0.63 and 0.65 for thoracic duct lymph of two normal anesthetized dogs.

The close correspondence of the ratios of protein of lymph to protein of serum in these series of observations suggests that for a given level of

the serum protein concentration the protein content of the lymph may vary only within certain fairly definite limits, the lower and upper levels of which are, for the normal animal, probably indicated fairly accurately by the lymph:serum ratios of 0.30 and 0.70 respectively. As a corollary to this conclusion it follows that a greater variation in the concentration of the lymph can occur in an animal having a high serum protein content than in one having a low concentration of serum protein. For example, if a dog has a serum protein of 4 per cent the lymph protein is limited to the range between 1.2 per cent and 2.8 per cent, a total variation of 1.6 per cent. An animal with a serum protein of 9 per cent, on the other hand, could presumably form a lymph containing anywhere from 2.7 per cent to 6.3 per cent, a total variation of 3.6 per cent. It may therefore be assumed that the variability of the protein content of the lymph is exactly proportional to the serum protein concentration, the variation being 0.4 per cent of protein for each 1 per cent of serum protein.

Specific osmotic pressure. The figures in table 1 which show the osmotic pressure of colloids for one per cent protein are calculated by dividing the values for the total osmotic pressure of the serum and lymph respectively by the corresponding protein contents. These values of the "specific osmotic pressure" of protein vary from 32 mm. to 48 mm. of water. Contrary to the recent findings of Loewen, Field and Drinker (10) the figures for lymph are, with two exceptions, *lower* than the corresponding figures for serum. In general the difference between the figures for lymph and those for serum may be explained by the fact that the total protein concentration of the lymph is always lower than that of the serum. As pointed out by Krogh (9) the osmotic pressure per one per cent protein falls as the concentration of protein decreases. This author presents in graphic form the relation between these two factors as found by several investigators for human blood sera, the protein concentrations of which were varied by ultrafiltration on the one hand and by dilution with Ringer solution on the other hand. Points plotted onto this graph from my data for lymph and for serum fall within the expected area. Five of the seven values given by Loewen for dog serum also lie within the range of figures determined by these curves, but the figures presented by this author for cervical and thoracic duct lymph cannot be reconciled either with his own figures for dog serum or with my figures for lymph, or with any reliable values for human serum which have appeared elsewhere in the literature. His values for lymph range from 55 mm to 103 mm with an average of approximately 70 mm. of water per one per cent protein, as contrasted with values of 44 mm. to 59 mm. for serum. Furthermore the values obtained by this author for the specific osmotic pressure of lymph vary *inversely* as the protein content of the lymph; i.e., the higher the protein content of the lymph the lower is the osmotic pressure per one per cent of protein. Inasmuch

as the figures given by these authors represent the only data previously published on the colloid osmotic pressure of lymph it will be necessary to discuss their findings in detail in order to discover, if possible, the reasons for the marked discrepancy between their values and the values found in the present study for lymph from the lacteals.

Such an anomalous relationship between concentration and specific osmotic pressure of colloids, as found by these investigators for lymph from the thoracic duct and cervical regions, has been encountered previously, so far as I am aware, only in the case of albumin of the urine of patients suffering from nephritis or cardiac insufficiency (13). Pressures as high as 280 mm. of water for one per cent protein are reported from studies on the urine of such patients. It is assumed that the capillaries of the glomeruli exhibit a selective permeability to the proteins of the serum, allowing protein of small molecular size (and correspondingly high osmotic pressure per gram) to pass through, but retaining the larger molecules, especially the globulins, in the blood. That this assumption is probably correct is indicated by the fact that the serum of such patients, which according to this theory should retain only the larger protein molecules, shows a relatively low specific osmotic pressure as compared to the serum of normal individuals. Loewen assumes a similar selective permeability of the capillary walls to explain the high specific osmotic pressures of lymph samples from the thoracic duct and cervical trunks. As serum albumin consists mainly of smaller and osmotically more active molecules than serum globulin this author suggests that the high osmotic pressure of the lymph must be due to the presence in this fluid of relatively much more albumin than globulin. Unfortunately he does not present chemical analyses to support this assumption. From the data of table 1 it is clear that the albumin:globulin ratio of lacteal lymph is somewhat higher in most instances than the corresponding ratio for serum. However, it is very doubtful that the albumin-globulin ratio of thoracic duct or cervical lymph is ever high enough to account for the high specific osmotic pressures which have been reported. It is probable that all lymph contains a considerable amount of globulin. It is also probably that only a small fraction of the albumin of the serum is capable of exerting as high a specific pressure as has been observed for the case of albuminous urine, for it has been found that edema fluid, which often contains relatively high proportions of albumin, exhibits a specific osmotic pressure which is not higher than that of the blood plasma (13).

The only explanation which can be offered at the present time for the discrepancy between the previously reported figures and those submitted here is that the former do not represent true equilibrium values. Although the authors refer to the difficulties of the method, especially the preparation of suitable membranes, they do not publish the criteria which enabled

them to decide when an osmotic equilibrium had become established. According to the criteria we have laid down in a preceding paper (6) on the method of determining the osmotic pressure of colloids, which criteria have been rigorously followed throughout the present series of observations, it is found that a much longer time is often required for the attainment of a true osmotic equilibrium in the case of lymph than in the case of serum. This delay may be attributed to the presence of fat, which tends to decrease the permeability of the membrane. The fat content of the lymph from most of my animals was not especially high, for they were fed on skimmed milk. But in a control experiment (described in the section on methods) and in several preliminary trials of the method of collection of lymph, the animals received cream, and the lymph contained a relatively large amount of fat, as was evidenced by the great difficulty encountered in the digestion of the sample with sulphuric acid in the determination of the nitrogen content. The determination of the osmotic pressure of such samples of lymph usually required more than 12 hours as compared with the usual time of 4 to 5 hours required for the attainment of equilibrium for lymph of low fat content, and 1 to 3 hours for serum. The suggestion which has been recently made (14) that lipids in a state of dispersion as they occur in serum during lipemia may in themselves be responsible for an osmotic effect was tested out, so far as the fat content of lymph is concerned, in the case of a sample with high fat content which was taken from the thoracic duct. The osmotic pressure per gram of protein was 44.4 mm. of water which is not higher than can be accounted for by the protein content of 4.07 per cent and the albumin:globulin ratio of 1.7. It is therefore extremely doubtful whether fat can exert an appreciable effect on the equilibrium osmotic pressure of colloids, even though it may undoubtedly delay the attainment of the true osmotic equilibrium.

It is well known that the initial pressure which is exerted by serum against an outer solution of salts is considerably higher than the equilibrium osmotic pressure. The excess of pressure is presumed to be due to non-protein substances which penetrate through the membrane with difficulty. Any condition, such as the presence of fat on the membrane, which delays the diffusion of these substances will prolong the period of excessive pressure. With slow membranes it is often exceedingly difficult to observe the gradual fall of pressure as these substances diffuse slowly into the outer solution. The process may require hours, days or weeks, depending on the permeability of the membrane, its thickness, etc. Readings of the osmotic pressure under such conditions are apt to be too high by a constant figure which will be proportional to the concentration of these slowly diffusing substances, other conditions being the same. Presumably the concentrations of these materials will be about the same in all similar samples of biological fluids from normal animals. Therefore the error in

the values of the colloid osmotic pressure which may appear as the result of neglecting the temporary pressures of these substances, will be a relatively constant error. The occurrence of a constant error in a series of values will produce a relatively large error in the calculated specific osmotic pressure for low protein contents and a relatively small error in the corresponding calculated values for high protein contents. In fact, it becomes possible to recalculate the figures obtained by the above-mentioned authors, allowing for a constant error, and thereby to obtain figures for the specific osmotic pressure which *increase* in a normal manner as the protein content of the lymph samples increases.

Whatever may be the reason for the great discrepancy between the osmotic pressure values for lymph which have previously been reported as compared with those presented in table 1, it is believed that the latter values are as nearly correct as can be obtained by a method in which rigid attention to details of the preparation and standardization of membranes and a close adherence to the necessary criteria for the estimation of the true equilibrium pressure have been practiced.

Total osmotic pressure. In the last column of table 1 are presented the figures for the total osmotic pressures of colloids of serum and of lymph. The bracketed figure was calculated from the total protein content of the lymph on the assumption that the specific osmotic pressure was 40 mm. water.

Relation between effective osmotic pressure and mesenteric venous pressure. Figure 1 presents in graphic form the relations found to exist between the "surplus" osmotic pressure of the blood (i.e., the osmotic pressure of colloids of serum *minus* the osmotic pressure of colloids of lymph) and the mesenteric venous pressure. It is probable that the surplus osmotic pressure is, in the case of the intestine, a fairly accurate measure of the "effective" osmotic pressure of the blood. This assumption is based on the fact that the flow of lymph from the intestine is continuous, at least during digestion. In the words of Krogh, Landis and Turner (15) "The more rapid the filtration . . . the more nearly will both average tissue fluid and lymph resemble the fluid filtered through the capillary wall." It therefore seems justifiable to assume that, on the average, the walls of the intestinal capillaries are permeable to the same fraction of the total concentration of plasma proteins as is found in the lymph from the lacteals, and that the surplus osmotic pressure is in reality of the same magnitude as the effective osmotic pressure. How then can we account for the fact that the effective osmotic pressure is as high as, or even higher than, the mesenteric venous pressure? Krogh (9) assumes that the mesenteric venous pressure is only slightly lower than the pressure in the intestinal capillaries. If this were true it would require a much lower effective osmotic pressure than has been calculated from the experiments of this series to allow a continuous

formation of lymph by filtration through these capillaries. Appropriate low figures for the effective osmotic pressure would require that the osmotic pressure of lymph be considerably higher than I have found it to be. The difficulty disappears, however, when we recall the recent observations of Landis (16) who found that the pressures in the capillaries of the mesentery of the rat and the guinea pig are considerably higher than the mesenteric venous pressure. For example, corresponding to a mesenteric pressure of 120 mm. water the arteriolar capillary pressure was 220 mm. and the venous capillary pressure was 150 mm., giving a mean

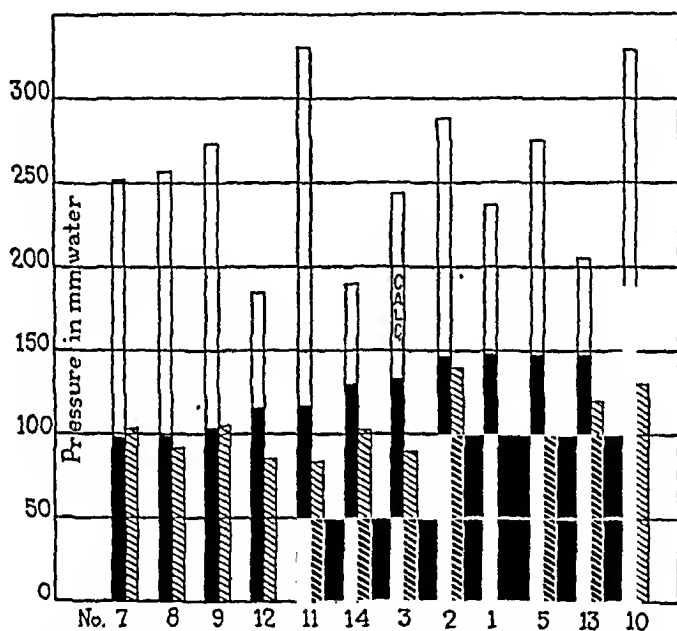


Fig. 1. Relation between the "surplus" osmotic pressure of serum and the mesenteric venous pressure. Data arranged by experiments in ascending order of the surplus osmotic pressures. Total height of black and white columns: Osmotic pressure of colloids of serum. Height of white columns: osmotic pressure of colloids of lymph. Height of black columns: difference between osmotic pressures of serum and lymph = surplus osmotic pressure of serum. Cross-hatched columns: lateral pressure in mesenteric vein.

capillary pressure of 185 mm. water. Although no measurements of the pressure have been carried out on the capillaries of the intestinal villi it is very probable that these pressures are considerably higher, perhaps 50 per cent higher, than the mesenteric venous pressures. If this is the case it becomes easy to understand how filtration may proceed continuously even when opposed by the relatively high effective osmotic pressures calculated from the data of these experiments.

Although the conclusion that lymph formation in the intestine occurs

as a result of capillary filtration appears to be valid, it cannot be definitely established until direct measurements of the capillary pressure in this region can be carried out.

SUMMARY

1. A method for the collection of intestinal lymph from the mesenteric lacteals of the dog is described.

2. The concentration and osmotic pressure of the proteins of this lymph as well as of blood serum were determined in a series of 12 dogs, and the mesenteric venous pressure was measured in the majority of the experiments.

3. The albumin:globulin ratio of the lymph is somewhat higher in most instances than the corresponding ratio for the serum, a finding which indicates that the capillaries of the intestine exhibit a slight degree of selective permeability with respect to these protein fractions.

4. The ratios of the total protein content of lymph: total protein of serum vary from 0.3 to nearly 0.7. It is suggested that the protein content of the lymph of any individual dog is determined, within these limits, by the protein content of the serum of the animal. The absolute variation in each case is proportional to the protein content of the serum so that for each 1 per cent of serum protein the lymph protein may show a variation of 0.4 per cent.

5. The specific osmotic pressure of proteins is slightly lower for lymph than for serum, the difference being due, presumably, to the lower concentration of protein of the lymph. The recent finding, by others, of a higher specific pressure for lymph from the thoracic duct and cervical region than for the serum of the same animal is discussed.

6. The mesenteric venous pressure is either of the same magnitude or slightly lower than the calculated effective osmotic pressure of the blood. In view of the recent determinations by Landis of the steep pressure gradient in the capillaries of the mammalian mesentery, it is believed that the average capillary pressure in the intestine is sufficiently higher than the mesenteric venous pressure to account for the continuous formation of lymph by a process of filtration of a protein-containing fluid from the capillaries of the region.

BIBLIOGRAPHY

- (1) STARLING, E. H. 1909. *The fluids of the body*. London.
- (2) MARKOWITZ, C. AND F. C. MANN. 1931. *This Journal*, xcvi, 709.
- (3) WELLS, H. S. 1932. *This Journal*, xi, 434.
- (4) FOLIN, O. AND C. J. FARMER. 1912. *Journ. Biol. Chem.*, xi, 493.
- (5) HOWE, P. E. 1921. *Journ. Biol. Chem.*, xlix, 109.
- (6) WELLS, H. S. 1932. *This Journal*, xi, 409.
- (7) MUNK, I. AND A. ROSENSTEIN. 1891. *Virchow's Arch.*, cxxiii, 230.

- (8) MORAWITZ, P. 1906. Beitr. z. chem. Physiol. u. Path., vii, 153.
- (9) KROGH, A. 1929. The anatomy and physiology of capillaries, revised. New Haven.
- (10) LOEWEN, D. F., M. E. FIELD AND C. K. DRINKER. 1931. This Journal, xcvi, 70.
- (11) MEYER-BISCH, R. AND F. GÜNTHER. 1925. Pflüger's Arch., ccix, 81.
- (12) ARNOLD, R. AND L. B. MENDEL. 1927. Journ. Biol. Chem., lxxii, 189.
- (13) IVERSEN, P. AND F. NAKAZAWA. 1927. Biochem. Zeitschr., xcvi, 307.
- (14) FISHBERG, E. H. 1929. Journ. Biol. Chem., lxxxi, 205.
- (15) KROGH, A., E. M. LANDIS AND A. H. TURNER. 1932. Journ. Clin. Invest., xi, 63.
- (16) LANDIS, E. M. 1930. This Journal, xciii, 353.

THE PASSAGE OF MATERIALS THROUGH THE INTESTINAL WALL

II. THE OSMOTIC PRESSURE OF THE COLLOIDS OF LYMPH FROM THE LACTEALS AS A MEASURE OF THE ABSORBING FORCE OF THE INTESTINE

HERBERT S. WELLS

From the Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee

Received for publication May 7, 1932

From the experiments (1) on the absorption of water from physiological saline solution placed in isolated jejunal loops of dogs it has been previously shown that the intestine exhibits a definite absorbing force, and that this force is accurately measured by the extent to which the intra-intestinal pressure must be lowered below atmospheric in order to prevent the absorption of fluid. It was suggested that the absorbing force may reside in the osmotic pressure of colloids of the tissue fluids of the villi, and it was urged that this hypothesis be tested by making a comparison, in individual animals, of the magnitude of the absorbing force with that of the osmotic pressure of the lymph from the lacteals.

From the data to be considered in the present communication it appears that the osmotic pressure of the proteins of the lacteal lymph is of the same magnitude as the absorbing force of the intestine. This conclusion is reached after consideration of data obtained from a series of 14 experiments on dogs in the course of which determinations were made of the osmotic pressure of colloids of blood serum and of lymph from the lacteals; of the total protein, albumin, and globulin content of lymph and of serum; of the mesenteric venous pressure; and of the absorbing force of the jejunum. The animals were anesthetized with barbital. With the exception of those findings which relate most directly to the topic under discussion, the data resulting from these experiments have been presented in detail in the preceding paper in THIS JOURNAL (2) and have there been analyzed in relation to the question of the mechanism of lymph formation in the intestine.

It was realized from the beginning of the investigation that the validity of any conclusions which might be drawn from the comparison of the osmotic pressure of lymph with the absorbing force would rest, first of all on the absolute degree of accuracy of the measurement of these two factors,

and secondly on the validity of the assumption that the osmotic pressure of the lacteal lymph is approximately the same as that of the tissue fluids which bathe the inner surface of the epithelial membrane of the intestine. The absorbing force may be determined with a considerable degree of accuracy. Measurements of the osmotic pressure of colloids in biological fluids are, however, always subject to possibilities of error. It has therefore been necessary to develop a method for the measurement of the osmotic pressure and, no other standard being available, to compare the values obtained by this method with values obtained by other investigators. In order to make such a comparison it was essential to determine the concentration of total protein and of the albumin and globulin fractions of the fluids, for the osmotic pressure of colloids is determined mainly by these constituents. The necessity for carrying out the analyses was further indicated by the fact that Loewen and his colleagues in Drinker's laboratory (4), who are the only other investigators to report figures for colloid osmotic pressure of lymph, found values which present marked variations in the relation of the pressure to the protein content and are also much higher than would be expected from the total protein content of the samples. These authors suggested that the relatively high values were due to the presence in lymph of a relatively high concentration of albumin. The results of my studies, which have been presented in the two preceding papers, indicate that the irregular and relatively high values found by these authors cannot be explained on the basis of the protein analyses of lymph and therefore are probably not true equilibrium values. These studies have indicated further that the method employed in this investigation does give true equilibrium values, that these values bear the same general relation to the protein content that are in evidence for corresponding samples of serum, and that the values for blood serum obtained by the same method correspond very well with the most reliable figures for the colloid osmotic pressure of serum which have been published by workers in other laboratories. It is therefore believed that the method provides results which are sufficiently close to the absolute values to allow a comparison to be made of the osmotic pressure of lymph with the absorbing force.

As regards the identity of lacteal lymph and the tissue fluids of the villi, it must be admitted that our knowledge of the mechanism of formation of lymph is inadequate to answer the question in an entirely satisfactory manner. It may be presumed, however, that the rapid flow of lymph which occurs in the intestine especially during digestion will, in the first place, favor the attainment of equilibrium between the protein concentration of the lymph and that of the average tissue fluids and, in the second place, will tend to insure that the lymph, in its passage from the villi to the lacteals of the mesentery, will have undergone minimal changes in composition. In the preceding paper (2) the results of a comparison of

the mesenteric venous pressure with the calculated effective osmotic pressure of the blood lend further support to the assumption that the tissue fluids of the villi are being constantly renewed as a result of the continuous filtration which must occur in this region. It should be emphasized that, in the controversy which has recently arisen in regard to the possibility that the tissue fluids can contain protein in high concentration, the special case of the intestine has always been excepted, for it is generally agreed that the capillaries in this region are relatively more permeable to protein than the capillaries elsewhere in the body, with the possible exception of the liver.

METHODS. The experimental methods, with the exception of the procedure for the determination of the absorbing force, have been described in detail in the two preceding papers, the first of which (3) is given over entirely to the method for the measurement of the osmotic pressure of colloids.

The determination of the absorbing force of the intestine. The determination of the absorbing force was carried out immediately after the lymph had been collected. The same general method was employed as was described in the first paper of the series (1). However, in spite of the fact that it has been found for the case of fasting dogs that the absorbing force remains very nearly constant over long periods of time, it was thought that with the changes which might occur in the digestive state it would be advisable to make as rapid a determination as possible. Consequently no attempt was made to plot the absorption curves. The intra-intestinal pressure was lowered at once to a level which might be expected to nearly abolish absorption. By shifting the pressure above and below this point it was usually possible, in the course of from one to three hours, to find a point at which absorption could just be detected and also to find a second point at a somewhat lower pressure, where "negative absorption" would occur. In most of the experiments the absorbing force of the gut could be determined within a fairly narrow range of pressures. The extent of the range of pressures within which the critical pressure can be found to lie is dependent largely on the relative intensity of movements of the gut. Peristalsis was reduced to a minimum by maintaining the temperature of the room at 30°C. and by exposing the isolated loop to air saturated with water vapor at a temperature of 38°C. to 40°C. This was accomplished by the use of a box containing a temperature regulator and humidifying device, so adapted in shape that it could be placed over the abdomen of the animal.

The horizontal diameter of the lumen of the isolated loop was taken as the reference level for the measurement of the intra-intestinal pressure.

PRESENTATION OF DATA. Figure 1 presents in graphic form the results of all experiments performed to test the degree of correlation between the osmotic pressure of colloids of lymph from the lacteals and the absorbing

force of the jejunum. It will be noted that in eight out of the fourteen experiments, those numbered 13, 14, 12, 1, 10, 2, 7, and 9 in the order of arrangement, there is a relatively close correspondence between the values for these two factors. In two additional cases (experiments numbered 5 and 6) the correspondence is more apparent than real, for the upper limit of the absorbing force could not be determined, due to slight hemorrhage into the bowel. Experiment 8 presents results which, due to this same interfering factor, are of little value, except in so far as the close correspond-

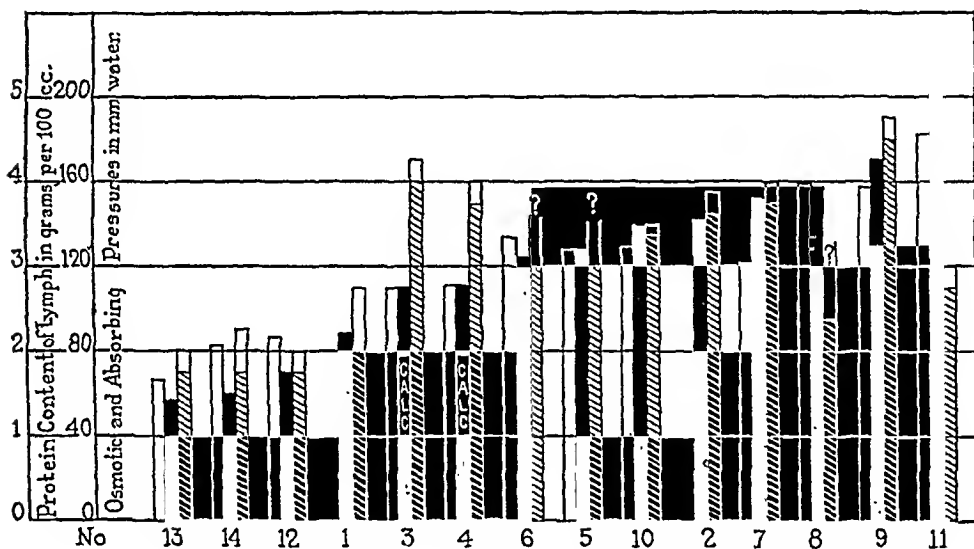


Fig. 1. Correlation of the osmotic pressure of colloids of lymph from the lacteals with the absorbing force of the intestine. The results are arranged in ascending order of the osmotic pressures of the lymph.

White columns: protein concentration of lymph in grams per 100 cc.

Solid black columns: Osmotic pressure of colloids of lymph in millimeters of water.

Cross-hatched columns with white tops: absorbing force of the gut in millimeters of water determined within the limits indicated by the heights above the base line of the base and top of the white area. The scale of protein concentration is so related to the scale of pressures that a one per cent protein content corresponds to an osmotic pressure of 40 mm. of water, which is not far removed from the average of all determinations. (See the previous paper (2) for the tabulated data.)

ence between the protein content and the osmotic pressure of the lymph is exemplified. In experiments 3 and 4 the osmotic pressures were not determined. The approximate values, calculated from the protein content of the lymph samples, are significantly lower than the corresponding values for the absorbing force of the gut. Experiment 11 presents, on the other hand, the single example in which the absorbing force is lower than the osmotic pressure of the lymph.

It should be pointed out that the occasional lack of agreement between

the osmotic pressure and the absorbing force might well be expected in view of the time (several hours) which necessarily elapsed between the collection of the lymph and the measurement of the absorbing force.¹ In this interval certain factors may occasionally operate to produce changes in the composition of the tissue fluids of the villi. In the first place a profuse (though usually transitory) secretion of intestinal juice occurs as the result of the mechanical stimulation due to the necessary manipulation of the gut. In the second place, the animal is in the digestive state and the whole intestinal tract is therefore in a condition of functional activity. By reason of circulatory or other changes associated with this activity the composition of the lymph may presumably change to some extent. It is obvious that the lymph taken from the lacteals of the mesentery may, in proportion to the extent of such changes, differ in composition from the lymph in the villi, which has been more recently formed. The fact that a rather wide range of protein concentrations of the lymph has been encountered in *different* animals has led to the conclusion (2) that variations can occur in the *same* animal within certain definite limits which are determined by the protein content of the serum of the individual animal. On the basis of such variations it becomes possible to account in a fairly satisfactory manner for the discrepancies, referred to above, between the osmotic pressure of the lymph and the absorbing force. Thus, in experiment 3 the ratio of the protein of lymph to protein of serum was 0.52. Assuming that previous to the determination of the absorbing force the ratio had risen to the upper limit of 0.64, there would have been an increase in the protein concentration of the lymph from 2.75 per cent to 3.4 per cent. The corresponding increase in the osmotic pressure would be sufficient to reduce the discrepancy between osmotic pressure and absorbing force by nearly 50 per cent. Similarly the discrepancies of experiments 4 and 11 can be accounted for *completely* without the necessity for assuming a relatively greater change in the protein content of the lymph for the individual animal than could be accounted for by the variations observed in the series of animals (2).

In the data presented in the first paper of this series (1) values for the absorbing force of the gut were found to lie between 80 mm. and 260 mm. of water. In subsequent experiments (unpublished) values as low as 40 mm. of water have occasionally been observed. In the series of experiments here reported the osmotic pressures of the lymph range from 57 mm. to 214 mm. of water. The failure to observe as high a value for the osmotic

¹ It has not seemed feasible to attempt to collect the lymph *after* the determination of the absorbing force, for, as the result of the operative procedures incident to the preparation of the isolated loop, the lymphatics of this loop as well as those of remaining parts of the intestine become constricted to such a degree that it would be very difficult to collect a sufficient amount of lymph from them.

pressure or for the absorbing force as was encountered in the first series of studies is doubtless to be attributed to the fact that the protein content of the sera of the dogs of this series was relatively low as compared to the normal range of protein concentrations for dog serum. Loewen (4) encountered a serum concentration of 8.84 per cent, with a corresponding value for thoracic duct lymph of 4.93 per cent. Allowing an osmotic pressure per one per cent protein of 47.8 mm. water (the highest value encountered for lymph in the present series) this lymph would have had an osmotic pressure of 236 mm. water. Meyer-Bisch and Günther (5) obtained from the thoracic duct of a normal dog lymph having a protein content of 7.2 per cent, with a correspondingly high value, 9.52 per cent, for the serum protein concentration. The osmotic pressure of this sample of lymph might well have been as high as 400 mm. of water. Although it is unlikely that the lacteal lymph will have a protein content as high as 7 per cent, under ordinary conditions, nevertheless it seems justifiable to assume that the absorbing force of the gut may vary within rather wider limits than have been encountered either in the present series of experiments or in the preceding investigations.

It has been pointed out (2) that the protein content of lymph from the lacteals appears to be fixed within definite limits by the protein content of the serum. Since the power of the intestine to absorb water resides in the osmotic pressure of the proteins of this lymph it follows that the absorbing force of the gut is fixed within definite limits by the protein content of the serum of the animal. This fact is related on the one hand to the general theory of the regulation of the water balance of the body and on the other hand it suggests certain possibilities in relation to the pathological physiology of some of the diseases in which the serum protein concentration is reduced below the normal level.

DISCUSSION. The osmotic theory of absorption having survived the critical test represented by the direct comparison of the absorbing force of the gut with the osmotic pressure of colloids of the lymph, it will now be desirable, on the one hand, to present as clear a picture of the process of absorption of fluid as is possible with the information at our disposal, and, on the other hand, to demonstrate that the osmotic theory can account for many of the observations concerning the absorption of fluid for whose explanation it has previously been considered necessary to invoke an active intervention of a "vital" force.

According to the osmotic theory the absorbing force of the intestine is due to the osmotic pressure, exerted against the semi-permeable epithelial membrane of the intestine, of that fraction of the total protein concentration of the blood serum to which, on the average, the blood capillary wall is permeable. This fraction of the serum proteins will pass by diffusion into any fluid which may be present between the capillary wall and the

epithelial wall of the gut, and diffusion will not be abolished, however much it may be retarded, by a flow of fluid in the opposite direction from the tissue spaces into the capillaries. The fact, however, that lymph appears to be formed continuously in the intestinal wall argues against the occurrence of any appreciable amount of absorption of fluid by the capillaries of this region and speaks rather for a constant and effective renewal of the protein-containing fluid in the tissue spaces of the villi by filtration from the capillaries. If one visualizes such a flow of fluid from the capillaries into the tissues and thence into the lymphatics one sees that it will provide for a certain amount of stirring of the fluids in the tissue spaces, a factor which is of great importance in the dynamics of osmosis. Since this stirring can be effective only in case the moving stream of fluid comes into actual contact with the osmotic membrane, it is of interest to note that the capillaries of the villus really are in close proximity to the epithelial wall. This proximity has formerly been considered as favoring the absorption of materials into the blood vessels and as placing the capillaries in a distinctly advantageous position, in this respect, as compared to the more distantly located central lacteal. It would now appear that the location of the capillaries close to the epithelium may rather be related to the maintenance of the absorbing force at a constant energy level.

It should be pointed out that materials which are dissolved in the absorbed water may readily pass by diffusion into the capillaries. Such diffusion may be retarded by the outward flow of fluid from the capillaries, but as this flow is probably not very rapid it cannot be expected to prevent the attainment of an approximate concentration equilibrium between the diffusible constituents of the tissue fluids and those of the blood. Additional provision for the attainment of such an equilibrium is perhaps furnished by the interchange of substances between blood and lymph which may be presumed to occur, due to the close proximity of thin-walled venules and lymphatic vessels in the submucosa (6). The disposal of the droplets of neutral fat, which are said to be synthesized in the villi from the absorbed fatty acid and glycerol components, may be a very complicated process but it seems highly probable that the majority of these relatively large particles will pass more readily along with the fluid stream and will enter the lymphatics, whose membranous lining is relatively permeable in comparison with the endothelium of the blood capillaries.

The picture of the process of absorption and disposal of various materials which has been presented above will account for many of the known facts. It should be emphasized, however, that the question of the disposal of material after it has been absorbed will not be completely solved until further knowledge concerning the mechanism of the exchange of fluids between the capillaries, tissue spaces and lymphatics becomes available. Absorption, on the other hand, at least as it relates to passage of *fluid*

through the epithelial lining of the gut, appears to be accounted for completely by the osmotic theory.

In this connection it should be mentioned that Starling, who first suggested a theory of absorption based on the osmotic pressure of colloids (7), was unable to accept his own suggestion by reason of certain facts which he considered provided evidence of an active intervention of the epithelial cells in the absorption process. An analysis of these objections, which have recurred again and again in discussions of the problem of intestinal absorption, reveals the fact that they do not rest on any firm basis of experiment and that, in truth, many of the observations which have been adduced as proof of the existence of a "vital" absorbing force can be more easily explained by the osmotic theory.

It is true that absorption studies have disclosed some very puzzling facts in relation to the selective absorption of various dissolved substances. Although further work along these lines is necessary it may be tentatively assumed that selective absorption has to do with selective permeability relationships between the membrane in question and the various molecular and ionic species which are presented to this membrane. It does not seem to be necessary, at present, to assume that the selection involves an expenditure of energy. Neither does the question of the absorption of *fluid*, with which the osmotic theory of absorption is concerned, enter, except very indirectly, into the problem of the selective absorption of dissolved substances.

The apparent irreciprocal or one-way permeability of the intestine with respect to certain substances, notably glucose, has offered another obstacle to the acceptance of a mechanical theory of absorption, although a careful analysis of the data of experiments which have been carried out to settle the question of the existence of such a one-way permeability forces one to the conclusion that no adequate test of the matter has yet been applied. In the absence of a single crucial experiment it will be best to leave the matter as it stands, realizing that the passage of dissolved substances through the gut wall may possibly involve the intervention of factors which are not concerned directly with the absorption of water.

The fact that water may be absorbed from a solution of sodium chloride which is slightly hypertonic with respect to the blood serum has been considered by Heidenhain, Starling and others as irrefutable evidence against a physical theory of absorption. Starling (7) demonstrates that theoretically the colloids of the blood could cause the complete absorption of such a solution, but only after it has become diluted by a preliminary passage of fluid into the gut until the intestinal contents have become isotonic with the blood. The fact that no such preliminary period is necessary, as is shown by the fact that the absorption of fluid from such a solution commences at once, forced Starling to the conclusion that we have to deal with

some peculiar intervention on the part of the epithelial cells—with a mysterious energy-consuming process, by which water may be transferred against an osmotic gradient. That this is unnecessary is indicated by the experiments of Lazarus-Barlow (8) who has shown that fluid can pass by osmosis from a solution of higher osmolar concentration to a solution of lower osmolar concentration provided the latter solution contains molecules which are either relatively or absolutely non-diffusible through the osmotic membrane which separates the two solutions. For example, fluid was observed to pass into ox serum from solutions of sodium chloride varying, in different experiments, from 1.5 to 2.0 per cent. The solution was separated from the serum by a peritoneal membrane. The concentrations of sodium chloride from which fluid might be removed by such “negative” osmosis are slightly higher than the concentrations at which the similar phenomenon of immediate absorption of hypertonic solutions from the gut may occur. The explanation of this “anomalous” process is obviously the same in both cases: the energy of diffusion of the more diffusible substance provides the energy for the transfer of water, in the direction of diffusion, against the osmotic gradient. This phenomenon has been carefully studied and its thermodynamic relation discussed by Shreinemakers (9). It is unfortunate that this possibility for the transfer of fluid against a gradient of osmolar concentration has not been more generally realized by physiologists. The failure to take such processes into account has contributed to many of the erroneous statements about the possibilities of the transfer of fluids which have appeared in the standard texts. For the present purpose it is only necessary to point out that the presence in the tissue fluids of the villi of the non-diffusing protein molecules makes possible, within certain limits, the absorption of “hypertonic” solutions of freely diffusible materials.

Another observation which has been taken as evidence for the activity of the cells during absorption is that isotonic sodium chloride solution will pass through the wall of a surviving intestinal membrane only in the direction from mucosa towards the serosa (10), (11). In these experiments, however, the protein content of the tissue spaces can account for the transfer of any amount of fluid in the above-mentioned direction, for there is no effective barrier to the passage of protein into the solution which is on the side of the serosa. The lymphatics are known to be permeable to protein. The capillaries, after the circulation has been interrupted, are probably almost completely permeable to this material. (But even if they remain impermeable it makes no essential difference in the development of the argument.) No impermeable membrane being present in the tissues of the gut wall to prevent the outward diffusion of protein, the whole gut wall, with the exception of the epithelium of the mucosa, becomes, in effect, a spongy mass of tissue with protein solution enclosed within its meshes.

The ligation of the larger lymphatics and blood vessels will not alter the situation. The protein solution within this sponge becomes, in fact, a continuous part of the outer solution of saline, with this qualification, that the bulk of the protein is held in close contact with the semipermeable epithelial membrane. If one should soak a thick layer of filter paper in blood serum and place it against one side of a collodion membrane which is semipermeable with respect to protein, and should place a solution of sodium chloride on either side it would require no invocation of a vital force to explain the resulting osmosis. The same reasoning applies to the case of the absorption, in one direction, of fluid through the excised skin of the frog (12).

The only experimental fact which is at all difficult to reconcile with the osmotic theory of absorption is that blood serum may be absorbed from the intestine. It is commonly assumed that the serum, under these conditions, will continue to exert its full osmotic pressure of colloids, but most investigators are agreed that the total amount of protein of the serum decreases to some extent, even though the concentration may be increased by the absorption of fluid. If this is true it would indicate very clearly that the protein is being broken down, presumably by the action of some digestive enzyme. If such digestion occurs it probably takes place most actively near the epithelial cells, so that one may easily suppose that the osmotic activity of the layer of serum nearest the cells is thereby abolished. It is possible, also, that other types of changes may take place in the serum which will reduce its osmotic activity. In no instance has the experiment been correctly controlled by an actual determination of the osmotic pressure of colloids of the serum remaining in the gut after the period of absorption. There is the further possibility that the absorption of fluid from serum may in part be due to increased intra-intestinal pressure. A further study of this problem is distinctly indicated but it is to be doubted that the presence of serum in the intestine can unmask a hidden mechanism for the absorption of water which has given no hint of its existence in the course of the investigations here reported.

It might well be emphasized at this point that the osmotic theory provides for the complete absorption of all fluid from the intestinal tract, unless this fluid contains in solution substances the particles of which are entirely unable to pass through the intestinal membrane, and which in addition can continuously exert an osmotic pressure sufficient to counterbalance the absorbing force of the proteins of the tissue fluids. The transformation of colloidal food materials into diffusible forms as the result of the processes of digestion is therefore to be considered not merely as a necessary preliminary step in the absorption of the food itself but as a process which may facilitate, at the same time, the absorption of the watery contents of the intestinal tract.

With the tentative exception of the absorption of water from serum the osmotic theory meets the main objections which have formerly been considered as sufficient grounds for ruling out any mechanical theory of absorption. The theory does not, however, attempt to evade the necessity for the expenditure of energy. This energy does not appear to reside in the epithelial cells nor in any other part of the intestine, at least under normal conditions, but is to be considered as being derived from those processes of the body which have to do with the maintenance of the osmotic pressure of the colloids of the blood. In so far as the amount and osmotic properties of the blood proteins and the condition of the capillary circulation in the villi remain unchanged, then, the potential of osmotic energy available for absorption of fluid can be maintained only by the elimination of water from the body. From a philosophical viewpoint, therefore, the "vital force," the Maxwellian demon (12), which has been invoked in the past to explain the intestinal absorption of fluid, is, as a consequence of these deductions from the osmotic theory, dispossessed from its residence in the enteric epithelium and exiled to other localities. The responsibility for the final eviction of this pest rests, henceforth, on those who are engaged in the study of the mechanisms by which water is excreted.

The elimination of the element of mystery from the absorption problem should encourage the further study of the regulatory mechanisms which, under normal and pathological conditions, are able to affect the direction and rate of transfer of water through the intestinal membrane.

SUMMARY

1. The osmotic pressure of colloids of lymph from the lacteals is found to correspond closely to the absorbing force of the intestine as measured by the negative intra-intestinal pressure necessary to abolish the absorption of fluid from isolated jejunal loops of dogs.

2. It is shown that, in general, the absorbing force is proportional to the protein concentration of the blood of the individual animal.

3. The findings indicate that the absorbing force of the intestine is due to the osmotic pressure, exerted against the semipermeable epithelial membrane of the intestine, of that fraction of the total serum proteins to which, on the average, the walls of the blood capillaries of the villus are permeable.

4. The probable relations of the process of formation of lymph in the intestine to the processes of absorption and disposal of fluid are considered.

5. The osmotic theory of absorption is subjected to the test of meeting the objections which have so often been raised against the acceptance of a physical theory of absorption of fluids.

6. From the osmotic theory it may be deduced that the energy potential of the absorbing force is maintained by those processes of the body which

govern the concentration of the blood proteins. In the normal animal these forces are remote from the intestine and are to be found in the organs of the body which have to do with the excretion of water.

BIBLIOGRAPHY

- (1) WELLS, H. S. 1931. This Journal, xcix, 205.
- (2) WELLS, H. S. 1932. This Journal, xi, 421.
- (3) WELLS, H. S. 1932. This Journal, xi, 409.
- (4) LOEWEN, D. F., M. E. FIELD AND C. K. DRINKER. 1931. This Journal, xcvi, 70.
- (5) MEYER-BISCH, R. AND F. GÜNTHER. 1925. Pflüger's Arch., ccix, 92.
- (6) KROGH, A. 1929. The anatomy and physiology of capillaries, revised. New Haven.
- (7) STARLING, E. H. 1909. The fluids of the body. London.
- (8) LAZARUS-BARLOW, W. S. 1896. Journ. Physiol., xx, 145.
- (9) SHREINEMAKERS, F. A. H. 1928. Journ. Gen. Physiol., xi, 701.
1929. Journ. Gen. Physiol., xii, 555.
- (10) COHNHEIM, O. 1899. Zeitschr. f. Biol., xxxviii, 419.
- (11) REID, E. W. 1901. Journ. Physiol., xxvi, 436.
- (12) REID, E. W. 1890. Journ. Physiol., xi, 312.
- (13) LEWIS, G. N. AND M. RANDALL. 1923. Thermodynamics and the free energy of chemical substances. New York.

THE RESPIRATORY QUOTIENT OF THE BRAIN¹

H. E. HIMWICH AND L. H. NAHUM

From the Department of Physiology, School of Medicine, Yale University, New Haven

Received for publication May 7, 1932

Although the respiratory quotient of the entire organism has been the subject of many investigations, the respiratory quotient of individual organs has not received the same exhaustive attention. However, the respiratory quotient has been determined for muscle (Himwich and Castle, 1927; Himwich and Rose, 1929; Doisy and Beckmann, 1922; Hines, Leese and Knowlton, 1931; Takane, 1926; Richardson, Shorr and Loebel, 1930); testicle (Himwich and Nahum, 1929; Shorr, Loebel and Richardson, 1930; Dickens and Simer, 1930, 1931); kidney (Richardson, Shorr, and Loebel, 1930; Dickens and Simer, 1930, 1931); and peripheral nerve (Fenn, 1927; Gerard and Meyerhof, 1927; Meyerhof and Schmitt, 1929). Loebel (1927) determined the respiratory quotient of cerebral tissue suspended in various nutritive media. A respiratory quotient of the brain in the living animal fixed at unity was reported by Himwich and Nahum (1929). The same value was obtained for excised brain tissue by Dickens and Simer (1930, 1931). Lennox (1931) working on human subjects obtained an average respiratory quotient of the brain of 0.95. The present paper contains the completed report of the respiratory quotient of brain observed in unanesthetized and amytalized dogs. The respiratory quotient was also determined after pancreatectomy, the injection of phlorhizin and various endocrine products; insulin, adrenalin, pituitrin, and pitressin.

METHOD. The brain of twenty amytalized dogs was studied after exposure of the superior longitudinal sinus and femoral artery so that blood samples could be drawn from both vessels practically simultaneously. Five unanesthetized animals: one normal, one injected with pituitrin and three with pitressin, were prepared under ether anesthesia a day or two previously to that on which the observations were made. Depancreatized dogs were usually examined 72 hours post-operative, the phlorhizinized animals after the D/N ratio had become constant. One animal was phlorhizinized after pancreatectomy. The endocrine extracts were injected subcutaneously and sufficient time was allowed to permit the full development of their actions before the blood samples were drawn.

¹ The expenses of this research were met in part by a grant from the Research Fund of the School of Medicine, Yale University.

The respiratory quotient of the brain was determined by analysis of samples of cerebral blood for carbon dioxide and oxygen content by the method of Van Slyke and Neill (1924). The carbon dioxide and oxygen capacity were also obtained after the blood had been exposed to a mixture of 5.5 per cent carbon dioxide in oxygen as described in previous reports (Himwich and Rose, 1929). Check determinations differed by 0.2 volume per cent or less. Previous to the withdrawal of the blood samples, expired air was collected and the respiratory quotient of the whole animal was determined by analysis of the expired air for carbon dioxide and oxygen with the Haldane-Henderson apparatus.

RESULTS. Table 1 contains a summary of the respiratory quotients of the normal, phlorhizinized, and depancreatized dogs, and table 2 illustrates the results of individual experiments. It may be seen that the respiratory quotients closely approximate 1 in each observation and that the average of 38 observations is unity. The average deviation of any respiratory quotient is ± 0.035 and the deviation of the mean is ± 0.006 . The average respiratory quotient of three observations of the brain of two dogs injected with insulin is 1.01 ± 0.03 . Two observations made on each of three dogs after injection of pitressin disclosed that in two dogs the respiratory quotients were not within the physiological range, probably the result of causes to be considered in the discussion. In the third animal, however, the respiratory quotients are 0.99 and 1.01. The brain of one dog receiving pituitrin has respiratory quotients of 1.08 and 1.02. Adrenalin like the pressor fraction of pituitrin affects the respiratory quotient, since in 7 of 10 observations of five dogs the respiratory quotient differed widely from unity.

In fifteen of eighteen observations the carbon dioxide capacity of the venous blood is greater than that of the arterial blood. The average increase is 0.74 vol. per cent. The average differences of the concentrations of the arterial and the venous blood are not significant. On eight occasions the venous blood is more concentrated disclosing an average increase of 0.34 vol. per cent and in nine other observations the venous blood is diluted on the average to the extent of 0.25 vol. per cent.

DISCUSSION. Results. That the respiratory quotient of the brain is unity has been substantiated by the results of Dickens and Simer (1930, 1931), obtained on brain tissue. The value of 0.95 observed on human brain by Lennox (1931) is in close agreement when one considers that this investigator obtained venous blood from the internal jugular vein which contains an admixture of blood from the brain and other parts of the head.

Foodstuffs utilized by the brain. The respiratory quotient of unity does not necessarily indicate that only carbohydrate is oxidized by the brain. It has been observed by Quastel² that glutamic acid is oxidized by excised

² Personal communication.

cerebral tissue. The respiratory quotient should be unity if the nitrogen atom forms urea. It is also possible that the oxidation of a combination of substances may yield a respiratory quotient of unity. For example, the respiratory quotient of succinic acid is 1.14 while that of protein without the formation of urea is 0.95. Moreover, succinic acid is readily oxidized by the brain (Quastel and Wheatley, 1931; Ashford and Holmes, 1931).

TABLE 1

NUMBER OF ANIMALS	CONDITION OF ANIMAL	NUMBER OF OBSERVATIONS	AVERAGE R. Q.
4	Normal	7	0.99
1	No anesthesia	4	1.04
4	Phlorhizinized	12	1.00
4	Depancreatized	11	0.99
1	Depancreatized and phlorhizinized	4	0.99
14		38	1.00±0.006

TABLE 2
Respiratory quotients of brain

CONDITION OF ANIMAL	AIR R. Q. OF INTACT ANIMAL	BLOOD R. Q. OF BRAIN
Fasted 2 days.....	0.75	1.03 1.01
Fasted 2 days; no anesthetic.....	0.77	1.06 0.97
Phlorhizinized.....	0.69	0.97 1.02
Depancreatized.....	0.67	0.94 1.00
Depancreatized and phlorhizinized.....	0.69	0.96 1.00 1.00 0.99

Richardson (1929) presents a list of various substances which have respiratory quotients close to unity. However, most of these substances, unlike glucose, appear in the blood in small concentrations. It is, therefore, probable that carbohydrate is the chief fuel of the brain.

In the normal animal (McGinty, 1929; Himwich and Nahum, 1929; and Nahum, Himwich and Koskoff, 1932) both glucose and lactic acid were

TABLE 3
Carbon dioxide and oxygen capacities of cerebral blood

DATE	KIND	CONDITION	CARBON DIOXIDE	OXYGEN
October 4, 1928.....	Arterial	Amytal	39.14	20.95
	Venous	Amytal	40.43	20.72
	Arterial	Amytal	40.15	21.18
	Venous	Amytal	39.78	21.28
November 1, 1928.....	Arterial	Amytal	40.61	14.82
	Venous	Amytal	41.83	15.46
	Arterial	Amytal	41.87	13.76
	Venous	Amytal	41.56	13.60
November 9, 1928.....	Arterial	No anesthesia	41.00	21.54
	Venous	No anesthesia	41.50	21.06
	Arterial	No anesthesia	41.22	
	Venous	No anesthesia	42.24	
November 10, 1928.....	Arterial	No anesthesia	42.00	19.47
	Venous	No anesthesia	42.94	19.40
	Arterial	No anesthesia	41.04	19.84
	Venous	No anesthesia	41.72	19.20
December 21, 1928.....	Arterial	Phlorhizin	37.07	20.94
	Venous	Phlorhizin	37.43	21.03
	Arterial	Phlorhizin	35.33	20.71
	Venous	Phlorhizin	36.34	21.00
November 15, 1928.....	Arterial	Depancreatized	41.40	22.31
	Venous	Depancreatized	41.79	22.63
February 14, 1929.....	Arterial	Depancreatized	37.69	18.50
	Venous	Depancreatized	37.51	18.63
	Arterial	Depancreatized	38.54	18.76
	Venous	Depancreatized	38.88	18.49
July 29, 1930.....	Arterial	Adrenalin	26.72	23.40
	Venous	Adrenalin	27.71	24.47
October 14, 1930.....	Arterial	Adrenalin	34.86	23.85
	Venous	Adrenalin	35.26	23.92
	Arterial	Adrenalin	32.01	24.07
	Venous	Adrenalin	32.14	23.92
January 11, 1932.....	Arterial	Pitressin	33.83	24.57
	Venous	Pitressin	34.88	24.37
	Arterial	Pitressin	31.90	23.84
	Venous	Pitressin	32.65	23.61

consistently absorbed from the blood by the brain. These substances are not changed to glycogen by the brain (Holmes and Ashford, 1930) nor does that organ store glycogen (Takahashi and Asher, 1925). According to Holmes (1930) the retained carbohydrate is oxidized by the brain but only after conversion to lactic acid for Holmes has demonstrated that cerebral tissue can utilize glucose only after transformation to lactic acid.

Glucose and lactic acid are removed from the blood by the brain of a diabetic animal (Himwich and Nahum, 1929; Nahum, Himwich and Koskoff, 1932). This glucose, however, cannot be oxidized directly for the respiratory quotient remains one even after pancreatectomy when the brain loses the insulin (Nothmann, 1925) necessary for the oxidation of glucose. It is therefore probable that the brain of the diabetic animal may also transform glucose to lactic acid before oxidation. Insulin is not required for the oxidation of lactic acid. Holmes and Holmes (1924) have found that the brain tissue of depancreatized cats oxidizes lactic acid.

It is evident that the respiratory quotient of the brain is not altered by insulin or pituitrin which are known to influence the character of the metabolism of the body as a whole. Adrenalin and pitressin, however, caused variations in the respiratory quotient of the brain not necessarily because of a change in the character of the foodstuffs oxidized but rather due to alterations in the acid-base, electrolyte and water equilibria. The acid-base equilibrium is shifted because of the production of increased amounts of lactic acid after injection of adrenalin (Tolstoi, Loebel, Levine and Richardson, 1924; Nahum and Himwich, 1931) or pitressin (Himwich and Fazikas, 1930; Bischoff and Long, 1931; Himwich, Haynes and Fazikas, 1932). In addition, adrenalin (Ederer, 1927; Haldi, Larkin and Wright, 1926) changes water and salt balances between tissues and blood, and pitressin probably is largely responsible for the effects of pituitrin on water and electrolyte (Underhill and Pack, 1923; Bushke, 1928) exchanges of the body.

The rise in the carbon dioxide capacity of the venous cerebral blood occurs despite the lower carbon dioxide capacity of the brain (Kleinschmidt, 1929) and is probably related to the constant removal of lactic acid from the blood passing through the brain. It is interesting to note that no change in the concentration of the cerebral blood is disclosed, probably due to the rapidity of the blood flow.

Method. Rapport (1930) has criticized the respiratory quotients of resting (Himwich and Castle, 1927) and exercising (Himwich and Rose, 1929) muscle obtained by the methods used in the present research because of possible sources of error due to differences in blood flow. Even admitting the possibility of sudden changes in blood flow during the withdrawal of the blood samples, the ratio of carbon dioxide produced and oxygen consumed would still be determined by the proportions of fat and carbo-

hydrate oxidized. The factors determining the accuracy of the method have been discussed previously (Himwich and Rose, 1929). They include an adequate gaseous exchange and the avoidance of changes in the acid-base and osmotic equilibria. In the present series of experiments these equilibria were disturbed only after the injection of adrenalin or pitressin. The gaseous exchange though not so large as in the experiments on exercising muscle was sufficiently great, approximately 10 vol. per cent both for carbon dioxide and oxygen and the average deviation of any quotient was ± 0.035 . Thus we see that by the observance of the proper experimental precautions each single respiratory quotient may be significant and an examination of table 2 reveals that most quotients are close to unity.

Oxygen consumption of the brain. With a cerebral blood flow of 140 cc. per 100 grams of tissue per minute (Jensen, 1904) and a removal of oxygen of 10 cc. per 100 cc. of blood (table 4), it may be calculated that the oxygen consumption is 14 cc. per 100 grams of brain per minute. This is much less than the oxygen used by the brain of smaller species for Holmes (1930)

TABLE 4

The average oxygen consumption of the brain and the entire organism

PREPARATION	NUMBER OF OBSERVA- TIONS	O ₂ REMOVED PER 100 CC. OF BLOOD	O ₂ CON- SUMPTION OF ENTIRE ORGAN	AVERAGE WEIGHT OF DOG
Amytalized and unanesthetized	11	10.99	83.38	15.64
Phlorhizinized	12	9.99	98.72	16.34
Depancreatized	11	9.40	113.8	17.33

found a value of 20 cc. per 100 grams of rabbit per minute. It is logical to suppose that the oxygen consumption of human brain may be less than that of the dog.

Alexander and Cserna (1912) reported an oxygen consumption of 36 cc. per 100 grams of brain of dog per minute. However, on recalculation of their results the oxygen consumption was found to be 13 cc. per 100 grams per minute, a value which is in close agreement with the present observations. Schmidt (1928) perfused the brain of dogs under various conditions and obtained results of the same magnitude.

The average brain weight of 70 grams in our dogs indicates a blood flow of approximately 100 cc. per minute. It can be seen from table 4 that the brain consumes about 13 per cent, 8 per cent, and 10 per cent of the entire oxygen intake of the body of normal, depancreatized and phlorhizinized dogs respectively. The average of the three values is approximately 10 per cent of the total oxygen consumption. Taking the average value it may be calculated that a respiratory quotient of 0.70 obtained from the expired air indicates that the average respiratory quotient of the body,

exclusive of the brain, is 0.67, while the respiratory quotients between 0.70 and 1.00 are diminished proportionately, 0.80 to 0.78 and 0.90 to 0.89.

SUMMARY

The respiratory quotient of the brain of 14 dogs (1 unanesthetized, 4 amytalized, 4 phlorhizinized, 4 depancreatized and 1 depancreatized and phlorhizinized) has been determined by analysis of the arterial and venous blood for carbon dioxide and oxygen by the method of Van Slyke and Neill. The average of the 38 respiratory quotients thus obtained was 1.00 ± 0.006 and the average deviation of a single result was ± 0.035 .

Four unanesthetized animals were studied after injection of pituitrin or pitressin and 7 dogs under amytal anesthesia were examined after injections of insulin or adrenalin. The respiratory quotient was not changed by insulin or pituitrin although it was by adrenalin and pitressin probably because of shifts in the water, electrolyte and acid-base equilibria.

Carbohydrate is constantly oxidized by the brain of the living animal. Since the respiratory quotient remains unity after pancreatectomy carbohydrate is not utilized in the form of glucose.

It has been calculated that the oxygen consumption of dog brain is 14 cc. per 100 grams of tissue per minute.

BIBLIOGRAPHY

- ALEXANDER, L. G. AND S. CSERNA. 1913. *Biochem. Zeitschr.*, liii, 100.
 ASHFORD, C. A. AND E. G. HOLMES. 1931. *Biochem. Journ.*, xxv, 2028.
 BUSCHKE, F. 1928. *Arch. Exp. Path. u. Pharm.*, cxxxvi, 43.
 DICKENS, F. AND F. SIMER. 1930. *Biochem. Journ.*, xxiv, 1301.
 1931. *Biochem. Journ.*, xxv, 985.
 DOISY, E. A. AND J. W. BECKMANN. 1922. *Journ. Biol. Chem.*, liv, 683.
 EDERER, S. 1927. *Arch. Exp. Path. u. Pharm.*, cxii, 211.
 FENN, W. O. 1927. *This Journal*, lxxx, 327.
 GERARD, R. W. AND O. MEYERHOF. 1927. *Biochem. Zeitschr.*, cxc, 125.
 HALDI, J. A., J. LARKIN AND P. WRIGHT. 1926. *This Journal*, lxxviii, 74.
 HIMWICH, H. E. AND W. B. CASTLE. 1927. *This Journal*, lxxxiii, 92.
 HIMWICH, H. E. AND J. FAZIKAS. 1930. *Proc. Soc. Exp. Biol. and Med.*, xxviii, 331.
 HIMWICH, H. E., F. W. HAYNES AND J. FAZIKAS. 1932. (In preparation.)
 HIMWICH, H. E. AND L. H. NAHUM. 1929. *This Journal*, lxxxviii, 680, xc, 389.
 HIMWICH, H. E. AND M. I. ROSE. 1929. *This Journal*, lxxxviii, 663.
 HINES, H. M., C. E. LEESE AND G. C. KNOWLTON. 1931. *This Journal*, xcvi, 50.
 HOLMES, E. G. 1930. *Biochem. Journ.*, xxiv, 914.
 HOLMES, E. G. AND C. A. ASHFORD. 1930. *Biochem. Journ.*, xxix, 1119.
 HOLMES, B. E. AND E. G. HOLMES. 1927. *Biochem. Journ.*, xxi, 412.
 JENSEN, P. 1904. *Pflüger's Arch.*, ciii, 171.
 KLEINSCHMIDT, E. E. 1929. *This Journal*, lxxxviii, 251.
 LENNOX, W. G. 1931. *Arch. Neurol. Psych.*, xxvi, 719.
 LOEBEL, R. O. 1925. *Biochem. Zeitschr.*, clxi, 219.
 MCGINTY, D. A. 1929. *This Journal*, lxxxviii, 312.
 MEYERHOF, O. AND F. O. SCHMITT. 1929. *Biochem. Zeitschr.*, ccviii, 445.

- NAHUM, L. H. AND H. E. HIMWICH. 1931. *Proc. Soc. Exper. Biol. and Med.*, xxix, 72.
- NAHUM, L. H., H. E. HIMWICH AND Y. D. KOSKOFF. 1932. (In preparation.)
- NOTHMANN, M. 1925. *Arch. Exp. Path. u. Pharm.*, cviii, 1.
- QUASTEL, J. H. AND A. H. M. WHEATLEY. 1931. *Biochem. Journ.*, xxv, 117.
- RAPPORT, D. 1930. *Physiol. Rev.*, x, 349.
- RICHARDSON, H. B. 1929. *Physiol. Rev.*, ix, 61.
- RICHARDSON, H. B., E. SHORR AND R. O. LOEBEL. 1930. *This Journal*, lxxxvi, 551.
- SCHMIDT, C. F. 1928. *This Journal*, lxxxiv, 223.
- SHORR, E., R. O. LOEBEL AND H. B. RICHARDSON. 1930. *This Journal*, lxxxvi, 529.
- TAKAHASHI, K. AND L. ASHER. 1925. *Biochem. Zeitschr.*, cliv, 444.
- TAKANE, R. 1926. *Biochem. Zeitschr.*, clxxi, 403.
- TOLSTOI, E., R. O. LOEBEL, S. Z. LEVINE AND H. B. RICHARDSON. 1924. *Proc. Soc. Exp. Biol. and Med.*, xxi, 449.
- UNDERHILL, L. P. AND A. T. PACK. 1923. *This Journal*, lxvi, 520.
- VAN SLYKE, D. D. AND J. M. NEILL. 1924. *Journ. Biol. Chem.*, lxi, 523.

STUDIES ON MAGNESIUM DEFICIENCY IN ANIMALS

II. SPECIES VARIATION IN SYMPTOMATOLOGY OF MAGNESIUM DEPRIVATION

ELSA R. ORENT,¹ H. D. KRUSE AND E. V. McCOLLUM

From the Biochemical Laboratory, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore

Received for publication May 9, 1932

When young rats are restricted to a ration containing only 1.8 parts per million of magnesium but adequate amounts of other dietary substances, they develop a characteristic and striking symptomatology that presages an early death. As was recently described in some detail (1), the animals pass successively through stages of vasodilatation, hyperirritability of the nervous system, cardiac arrhythmia, and fatal tonic-clonic convulsions. Furthermore, it was demonstrated at that time that magnesium deficiency per se is responsible for the syndrome; anorexia, inanition, or some unknown deficiency may be excluded from consideration. The evidence, in all, led to the conclusion that in at least one species magnesium is an essential element for normal functioning, growth and life.

As the symptomatology unfolded it became a matter of some interest to determine whether magnesium is indispensable to another species besides that represented by the rat. To that extent the necessity of the element for animal economy would be the more convincingly demonstrated. Further, by reference to the rat's behavior on a low magnesium diet as a standard it appeared important to ascertain whether lack of magnesium would be so severely manifested and whether the symptomatic pattern would be faithfully duplicated in another animal. Finally, it was recognized that the details of the pathogenesis of magnesium deficiency would be the more readily obtained from an animal more suited to metabolic studies than is the rat. Therefore dogs, representing a species seemingly appropriate for the purposes in mind, were restricted to the same diet as had been the rats. The ration was adequate in all respects except magnesium, of which it contained only 1.8 parts per million.

When young dogs, 6 to 7 weeks old and weighing 2.7 to 3 kgm., are limited to the magnesium-deficient diet, they exhibit like the rat a remarkable succession of symptoms which result always in death. Within two weeks the nail-beds, particularly of the forepaws, take on a flush and then

¹ National Research Fellow in Biological Sciences (Biochemistry) 1930-32.

in turn the tongue, eyelids, and buccal mucosa undergo the same change. By four to six weeks the ears become involved and the vasodilatation is complete; the distended vessels impart a florid appearance specifically to these skin areas. Then the vasodilatation gradually but entirely disappears, sometimes to recur at various intervals at a later time. The rubescence, the heightened color of the tongue and nail-beds from vascular engorgement, might suggest the presence of polycythemia vera. But there the correspondence ceases. The blood findings and course of the disorder are not indicative of polycythemia; for there is no erythrocytosis and the vasodilatation, after a comparatively short duration, is superseded by more baleful symptoms.

An extraordinary alteration occurs in the appearance of the extremities. The phalanges begin to spread apart, and the extremity from the region of the proximal end of the metatarsals downward increases greatly in size (fig. 1). The animal, instead of walking on its paws, bears much of its weight on its metatarsals; thereby the line of curvature of the nails, instead of projecting downward, arches out horizontally. Later the nails become so brittle that they break. These changes are particularly prominent in older animals that have survived on the diet for some length of time.

Loss of hair occurs generally over the body but is especially severe around the eyes, on the legs, tail, and abdomen, where small circumscribed areas may become completely denuded. Elsewhere what hair remains is dry and shaggy. The skin in the denuded areas of the abdomen, tail, and extremities undergoes erythematous changes, then desquamation, and may even go on to ulceration. Eventually the discharging ulcers may become dry and disappear, but usually fresh lesions appear nearby. All over the body the skin is dry and scurfy.

The ears, which were swollen and spongy during the hyperemic stage, become thick, leathery, and mottled; they even show old hematmata as evidence of previous hemorrhage. Lacrimal secretion may become persistent. The urine is deeply pigmented, but is normal in volume and free from blood.

After a month on the magnesium-deficient diet many but not all animals show evidences of increased irritability of the nervous system. That hyperexcitability does actually occur in some, if not all, cannot be doubted, because stimuli ignored by normal control animals are capable of releasing convulsions in the experimental group. For example, rattling of windows in a severe windstorm brought on seizures in several animals on the magnesium-poor ration, while control animals did not even manifest concern. Because the extent to which the irritability is heightened before rendering the experimental animals susceptible to convulsions is not always readily apparent, because the action of a stimulus cannot be predetermined, and

sometimes an exciting cause cannot even be discovered, and because abruptness and unexpectedness characterize the convulsive state, it became desirable to detect some sign which would infallibly indicate when the animal had reached the critical point in sensitivity. This objective was not attained. For a time we felt that rectal temperature would serve as a premonitory sign of impending convulsions; for in many instances a sharp drop in temperature was not uncommon several hours before a seizure.



Fig. 1. An animal, which had been deprived of magnesium for a considerable period of time, is shown with greatly swollen paws, which are much flattened, with widespread digits, and with horizontally arched nails.

This phenomenon, however, was not sufficiently constant to be reliable. Empirically we have found that if the dogs are started at an age of six weeks and a weight of 2.7 to 3 kgm., they usually have the first convulsion within five to seven weeks on the diet. Very often the animals have lost a slight amount of weight before the attack appears.

The pattern of the convulsions is remarkable for its constancy. The attack is ushered in without a cry. The susceptible animal, because of a disturbing noise or for no apparent reason, becomes restless and uneasy; it paces about the cage until it collapses on its side in convulsions. The

entire body, rigid from a tonic contraction, is thrown into a characteristic posture. The head is drawn back and the extremities are stiffly extended. The tongue, protruding slightly, may be perforated by clenched teeth. The skin is cyanotic. Respiration ceases during the attack and returns with muscular relaxation.

After a momentary subsidence of the tonic convulsion, tremors appear over the body and then clonic convulsions set in with the animal alternately flexing and extending its extremities. In flexion the fore extremities are drawn closely to the thorax with bending at the metacarpophalangeal joint. The eyeballs fluctuate in size. Respiration is rapid and shallow. This phase of the convulsive attack is not invariable but is usually seen.

After the tonic-clonic convulsions comes a period of exhaustion. From extreme muscular weakness the animal lies on its side, rarely attempting to rise. Lacrimal secretion drains from dull, shrunken eyes. There is discharge of nasal secretion, champing of the jaws, and drooling of regurgitated stomach contents from the mouth.

Usually the convulsive attacks follow one another serially with only brief intermissions during which the animal recovers from exhaustion. In one of these intervals the respiration, which had momentarily ceased during the phase of tonic contraction, is slowly returning to normal when it suddenly stops. After a short period of beating the heart likewise ceases. The animals always die during an exhaustion period when recovery is apparently in sight. In the initial attack the mortality is as high for dogs as it is for rats; 13 of 15 dogs, representing 86 per cent, succumbed during a recovery phase of the first series of convulsions.

In addition to the spectacular symptomatology which carries with it such a fatal outcome, the animals on the low magnesium diet show an interesting growth record (chart 1). The usual curve is one either of maintenance of weight, or slight decline; ordinarily a convulsive seizure takes off the animal before the loss is severe.

The degree of irritability of the nervous system, the weight record, and the length of the survival period depend largely on the extent to which the diet is deficient in magnesium, and the age and weight of the animal. Containing only 1.8 parts per million of magnesium, the diet which we employed is almost free from the element, so that the effects of its deficiency may be easily elicited. If it is desired, therefore, to produce the acute form of the disorder, the age and weight of the dogs become a most important consideration. In general, if animals are carefully selected within the age and weight range of 6 to 7 weeks and 2.7 to 3 kilograms respectively, the hyperexcitability is quite pronounced, the growth curve shows little rise, and the life span is short. If this detail is not minded and older dogs of heavier weight are placed on the diet, the deficiency is

always ultimately felt, but the growth record may be one of slight gain for a time and the survival period may be much prolonged.

It is certain that the foregoing symptomatology and growth record are due to a deficiency in magnesium, and not to inanition or the lack of an unknown substance. The animals on the magnesium-deficient ration give no evidence of diminution in food intake at any time. Further, the growth curves of control animals fed the magnesium-low diet plus 0.05373 per cent magnesium, added in the form of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, as contrasted with the records of the experimental animals on the magnesium-poor diet, leave

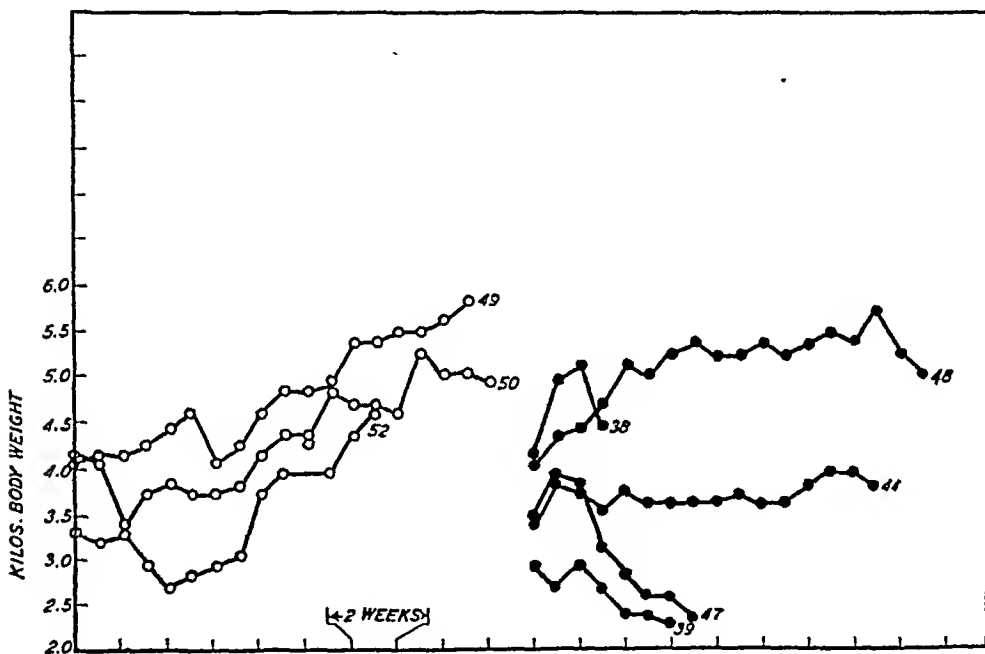


Chart 1. Weight curves of dogs. The curves with clear circles represent the weight records of dogs fed the magnesium-low diet plus added $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The curves with the solid circles represent the weight records of dogs fed the magnesium-low diet throughout life. These latter curves likewise show the survival periods of the animals; the final circle representing the weight just before a fatal convulsion.

no doubt that the latter suffered from a true magnesium deficiency (chart 1). The control animals, given the magnesium-low diet with added magnesium from the start, showed gain in weight and freedom from symptoms for eighteen weeks, at the end of which time the experiment was terminated.

TECHNIQUE. For production of an exceedingly acute deficiency, that gives a short survival period with reasonably consistent results, it is essential that dogs be selected with an initial weight of 2.7 to 3 kgm., at an age of 6 to 7 weeks. If older dogs are inadvertently used, in time the mag-

nesium deficiency will become just as manifest as in a younger dog, but the survival period is protracted.

In our experiments the dogs, although they showed no tendency to coprophagy, were confined in cages with screen bottoms. The animals had constant access to the diet and distilled water. The preparation of the magnesium-low diet, containing only 1.8 parts per million of the element, has already been described (1). For use with dogs we found it advisable to moisten the ration so that it could not be so readily scattered from the feeding pan. The control animals were given the magnesium-deficient ration plus 0.05373 per cent magnesium, added in the form of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

DISCUSSION. It will be recalled that we were interested in determining whether magnesium was indispensable to the dog, whether a deficiency in this component would be felt so severely, and whether the symptoms arising would follow substantially the same pattern as in the rat. That magnesium is essential to the dog is readily evidenced by the physiological disturbances, interruption of growth, and early death in the animals deprived of the element. By so much it becomes evident that magnesium is a nutrient necessary for animal economy.

Practically all the symptoms seen in the rat as the result of magnesium deprivation are likewise present in the dog consuming the same diet, so that qualitatively the deficiency is manifested in the same way in both species. Both show vasodilatation, hyperirritability, trophic disturbances, and generalized convulsions. Whatever variations appear between the symptomatology of the two species are slight, and are a matter of differences in intensity, time of appearance, and deviation. These slight differences are not surprising when the constitutional dissimilarities between the two species are taken into account. Furthermore, it must be remembered that the rats were offspring of a carefully controlled and uniform breed; whereas the dogs were of various breeds, and often the age was a matter of doubt.

The shades of intensity in symptomatology between the two species might well be mentioned in detail. In the dog, the vasodilatation does not stand out with quite the same prominence as in the rat; it is less intense and less generally distributed; the hyperirritability, while present in the dog, is not so apparent as in the rat; and the convulsions are less violent in the dog. The rat races at full speed just before going into convulsions, and his clonic contractions are much more vigorous and violent. On the other hand, the dog suffers much more in general nutrition and weight prior to the onset of convulsions than does the rat.

It is apparent that all these points deal with differences of degree such as would be expected from unlike species. We believe that one explanation is sufficient for all. Just as most other nutritional disorders give rise not only to constitutional effects, such as impaired nutrition, but also

local effects upon some system, so magnesium deficiency exerts an influence generally upon growth and weight, and locally upon the irritability of the nervous system. In humans there is a wide difference in the state of sensitivity of the nervous system in infants and adults; the nervous system of the former is inherently in a more irritable condition. It is not unreasonable to presume that such differences in sensitivity may arise from variations not only in age but also in species. Although no mensurable terms have ever been applied to contrast the nervous irritability of the dog with that of the rat, it is certain that differences must exist—by nature the rat is much more wild and excitable than the dog.

Accordingly, the normally irritable nervous system of the rat would suffer before the general effects of malnutrition would be felt. As a matter of fact, the young rats show vasodilatation and hyperexcitability, and die before nutritive loss sets in. On the other hand, the more stable nervous system of the dog does not fall under the localizing influence of the magnesium deprivation until the deficiency has made itself felt in malnutrition and loss of weight. Even here the general nutritive effect does not proceed far before increased irritability of nervous system leads to fatal convulsions. Actually then the results of magnesium deficiency are the same for the dog as for the rat, a general effect on nutrition and a local effect on the nervous system; but the relative prominence and order of appearance of the effects depend upon the inherent irritability of the nervous system in the two species. Whatever the slight differences in sensitivity between the two animals, it should not be forgotten that the influence on the nervous system is always felt and is always the cause of death in both.

Much the same reasoning may be applied in explaining why adult animals, both rats and dogs, show much more nutritive and trophic change before the onset of fatal convulsions, have their seizures at a later date, and survive so much longer than do young animals on the same magnesium-deficient diet. It may be that the greater magnesium store in the older animals enables them to withstand the deficiency for a longer time so that the tissues suffer in maintaining the magnesium of the blood at a normal level; that condition would explain the prominence of constitutional and trophic changes before the belated appearance of convulsions. It would seem more probable, however, that the greater susceptibility of the young animals to convulsions, which take them off before any striking nutritive changes occur, depends upon an inherently intensified irritability of the nervous system which is heightened the more by magnesium deficiency. By the same token, the nervous system of the adult animal possesses sufficient stability so that the whole body suffers from lack of magnesium as soon as or before the nervous system is sufficiently sensitive to respond with convulsions.

The dog, like the rat, manifests the effects of magnesium deficiency by the following symptom-complex: vasodilatation, hyperexcitability, and generalized convulsions. Regarded separately these symptoms might point to several conditions, but taken in association they are, we believe, indicative of tetany. In a previous publication (1) we stated at some length our reasons for viewing the syndrome as tetany. At that time we suggested that low-magnesium tetany was a distinct disorder, that vasodilatation was a vasomotor spasm, and that it was a characteristic feature differentiating low magnesium tetany from other forms, particularly the so-called infantile tetany. However, vasodilatation is not the only point of distinction between them. In the low-magnesium animals the absence of both laryngospasm and carpopedal spasm, which are so common in infantile tetany, is not without significance. Further study devoted to chemical changes in the blood and urine following magnesium deprivation may indicate additional points of difference.

SUMMARY

As in the rat, restriction of the dog to a diet containing only 1.8 parts per million of magnesium but adequate amounts of other dietary essentials leads to a symptom-complex of vasodilatation, hyperirritability of the nervous system, generalized convulsions, trophic changes, and nutritive failure. Although both species show all these symptoms, some are more prominent in the one than in the other. It is suggested that magnesium deficiency manifests itself generally by trophic disturbances and nutritive failure, and locally by increased irritability of the nervous system. In the dog the vasodilatation, hyperexcitability and convulsions are less intense while the trophic and nutritive changes are relatively more conspicuous than in the rat, because inherently the nervous system of the dog is more stable. In any event these differences are only a matter of degree because convulsions lead to death in both species. It is reiterated that the symptom-complex ensuing from a magnesium deficiency is tetany, differentiated from other forms principally but not solely by the vasomotor spasm.

BIBLIOGRAPHY

- (1) KRUSE, H. D., E. R. ORENT AND E. V. MCCOLLUM. *Journ. Biol. Chem.*, 1932, xcvi, 519.

GLYCOGENOLYTIC EFFECT OF EPINEPHRINE ON SKELETAL MUSCLE

S. G. MAJOR AND FRANK C. MANN

From the Department of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minnesota

Received for publication May 12, 1932

The conflicting data concerning the effect of epinephrine on glycogen in muscles may be accounted for by the fact that various investigators have removed the specimens of muscle under the influence of different anesthetic agents, or because different amounts of the substance have been injected into the body by different routes. Our investigations were undertaken to determine the effect of epinephrine on glycogen in the muscle under the influence of an anesthetic agent which has very little, if any, glycogenolytic action. Furthermore, the rate of injection was carefully controlled.

LITERATURE. The literature dealing with the effect of epinephrine on glycogen is too extensive to be reviewed here. Consequently only references pertinent to the subject will be mentioned.

Eadie (1929), working on cats under the influence of iso-amylethyl barbiturate (amytal), found that the glycogen in muscles was not significantly reduced one and a half hours after the subcutaneous injection of 1 mgm. of epinephrine. Firor and Eadie found specimens of muscle from cats under iso-amylethyl barbiturate taken an hour after the administration of epinephrine did not differ appreciably in glycogen content from those of the control series. On the contrary, Eadie (1929) found that after the subcutaneous injection of 0.1 mgm. of epinephrine to white rats a decrease of glycogen occurred, although this was not observed when larger doses were given. He was of the opinion that the difference between the rat and the cat, in regard to the reaction of epinephrine is a difference of species, and is not due to variations in dosage or in time of sampling. Soskin stated that the depletion of glycogen in muscles subsequent to injection of epinephrine, noted by many workers, may be accounted for by increased muscular activity. Houssay and Mazzocco found the glycogen content of suprarenalectomized rats to be lower than that of a control group.

In favor of the decrease of glycogen in muscles due to the injection of epinephrine, the following evidence may be cited: Blatherwick and Sahyun found that after the subcutaneous injection of 1 mgm. of epinephrine the

glycogen of muscles of rabbits was diminished. Agadschanianz found that glycogen disappeared completely from the muscles of fasting dogs which had received from 1.1 to 1.5 mgm. of epinephrine. Cori and Cori have demonstrated a constant decrease of glycogen subsequent to the administration of epinephrine. They also found that following such injections the lactic acid level of the blood was markedly elevated. Ohara found that the subcutaneous injection of 0.5 to 1 mgm. of epinephrine was followed by marked reduction in glycogen. Pollak noted that after repeated injections of epinephrine into rabbits glycosuria ceased and that the glycogen content of the skeletal muscles was reduced almost to zero. Ringer, Dubin and Frankel demonstrated that after the administration of epinephrine to phlorhizinized dogs the "residual glycogen" disappeared, and thus they were able to rid the muscles of glycogen. Sahyun and Luck stated their belief that following injection of epinephrine there is an initial period in which hyperglycemia develops and in which the liver and glycogen in muscles diminish, whereas in the latter phase of the action of the substance the glycogen in muscles continues to decrease, and the glycogen in liver increases. Ohara injected epinephrine intravenously into dogs with Eck fistulas, into head-thorax preparations and into dehepatized animals. In each group he obtained a rise in sugar in the blood and a decrease in glycogen in the muscles. Geiger and Schmidt found that injection of epinephrine into phlorhizinized animals causes decrease in glycogen in muscles and an increase of the lactic acid of the blood, the glycogen in muscles being transformed to lactic acid, and the latter in turn being converted into glycogen in the liver. Sachs demonstrated a slight but definite diminution of glycogen in muscles following administration of epinephrine.

METHODS AND MATERIALS. All experiments were performed on dogs under the influence of iso-amylethyl barbiturate. Previous work had demonstrated change in glycogen of muscles is only slight under this form of anesthesia (Major and Bollman).

Best, Hoet and Marks have noted that corresponding muscles of opposite extremities correspond closely in glycogen content. This observation has been repeatedly confirmed by Markowitz and Soskin, and we were able to confirm it also. Consequently, when experimental procedures were carried out on the glycogen content of a muscle, its fellow was used as a control, the control muscle being secured prior to subjecting the animal to the procedure in question. In a few cases a section of the same muscle was used as the control, the greatest care being taken to preserve the blood supply, the usual procedure being to use the distal end of the muscle as the control, the proximal end thus being insured a good supply of blood.

The epinephrine was injected intravenously in all the experiments, since it was felt that if injected subcutaneously the rate of absorption might not be uniform. The epinephrine was diluted to such an extent that 1.5

cc. entered the vein each five minutes of the experimental period in cases in which the drug was given slowly, the solution running into the vein by gravity from a graduated buret. In a few of the early experiments larger doses of epinephrine were injected quickly.

Glycogen was determined according to the method described by Pflüger, with slight modification.

RESULTS. *Effect of iso-amylethyl barbiturate on glycogen in muscles.* Although the influence of this anesthesia on glycogen in muscles was reported in a preceding paper it should be noted here that iso-amylethyl barbiturate has little, if any, effect on the glycogen content of skeletal muscle. A representative protocol follows.

From 8:35 a.m. to 8:50 a.m. 40 mgm. of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously into a dog weighing 22.5 kgm. The control specimens of muscle were removed from 9:09 a.m. to 9:15 a.m. At 10:30 a.m. eight additional milligrams of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously. The second set of specimens of muscle was removed from 11:30 a.m. to 11:34 a.m. Results were as follows:

MUSCLE	TIME	GLYCOGEN
		<i>per cent</i>
Right sartorius.....	9:09 a.m.	0.259
Left sartorius.....	11:30 a.m.	0.225
Right quadriceps.....	9:11 a.m.	0.376
Left quadriceps.....	11:31 a.m.	0.363
Right gracilis.....	9:14 a.m.	0.223
Left gracilis.....	11:32 a.m.	0.225
Right adductor.....	9:15 a.m.	0.329
Left adductor.....	11:34 a.m.	0.384

Effect of massive doses of epinephrine on glycogen in muscles. Five experiments were performed to determine the effect of large doses of epinephrine on glycogen in muscles. The doses varied from 1 to 2.5 mgm. each hour, over periods ranging from three to eighteen hours. In all such cases it was found that the glycogen content of the muscles was definitely diminished. Only one such experiment is given in detail, but it is typical of all those of this series.

From 8:20 a.m. until 8:50 a.m. 47 mgm. of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously into a dog weighing 19 kgm. After taking a set of specimens of muscle, which served as controls, epinephrine was injected intravenously continuously at the

rate of 2.5 mgm. each hour for a period of two hours. The results were as follows:

MUSCLE	TIME	GLYCOGEN
		<i>per cent</i>
Right sartorius.....	9:47 a.m.	0.439
Left sartorius.....	12:00 m.	0.270
Right adductor.....	9:46 a.m.	0.813
Left adductor.....	12:08 p.m.	0.728
Right gracilis.....	9:45 a.m.	0.515
Left gracilis.....	12:05 p.m.	0.299
Right quadriceps.....	9:44 a.m.	0.625
Left quadriceps.....	12:04 p.m.	0.420

Effect of rapid injection of 0.04 mgm. of epinephrine. To determine the effect of the rapid injection of 0.04 mgm. of epinephrine two experiments were performed. The results were similar, although different in the degree of glycogenolysis. In the first experiment, which is not given in detail, the glycogen was diminished 50 per cent in four hours. The second experiment is reported below. The dog used in this experiment weighed 23 kgm. and was under iso-amylethyl barbiturate anesthesia. After taking the first set of specimens of muscle the epinephrine was injected over a five-minute interval, from 11:15 a.m. until 11:20 a.m. Subsequently two other groups of specimens of muscle were taken. The results were as follows:

MUSCLE	TIME	GLYCOGEN
		<i>per cent</i>
Left sartorius.....	10:55 a.m.	1.020
Right sartorius.....	11:45 a.m.	0.576
Left sartorius.....	12:07 p.m.	0.689
Left quadriceps.....	10:56 a.m.	0.809
Right quadriceps.....	11:47 a.m.	0.664
Left quadriceps.....	12:08 p.m.	0.548
Left gracilis.....	10:57 a.m.	0.417
Right gracilis.....	11:49 a.m.	0.362
Left gracilis.....	12:10 p.m.	0.473
Left adductor.....	10:58 a.m.	1.350
Right adductor.....	11:51 a.m.	1.230
Left adductor.....	12:11 p.m.	0.693

Effect of rapid injection of 0.02 mgm. of epinephrine for each kilogram of body weight. Four experiments were performed using 0.02 mgm. of epinephrine for each kilogram of body weight. In all the decrease in glycogen in the muscles varied only slightly. The following is typical of the group.

The dog weighed 22.3 kgm. After anesthesia had been induced two control sets of specimens of muscles were removed. At 9:53 a.m. 0.02 mgm. of epinephrine for each kilogram of body weight was injected quickly by the intravenous route, with results as follows:

MUSCLE	TIME	GLYCOGEN
		<i>per cent</i>
Right sartorius.....	9:21 a.m.	0.817
Right sartorius.....	9:45 a.m.	0.838
Left sartorius.....	9:58 a.m.	0.793
Right sartorius.....	10:27 a.m.	0.669
Left sartorius.....	10:52 a.m.	0.707
Left sartorius.....	12:17 p.m.	0.647
Right adductor.....	9:23 a.m.	0.979
Right adductor.....	9:52 a.m.	0.957
Left adductor.....	10:14 a.m.	0.907
Right adductor.....	10:33 a.m.	0.829
Left adductor.....	10:56 a.m.	0.808
Left adductor.....	12:22 p.m.	0.839
Right quadriceps.....	9:24 a.m.	0.825
Right quadriceps.....	9:47 a.m.	0.846
Left quadriceps.....	10:12 a.m.	0.746
Right quadriceps.....	10:29 a.m.	0.754
Left quadriceps.....	10:52 a.m.	0.569
Left quadriceps.....	12:20 p.m.	0.501
Right gracilis.....	9:26 a.m.	0.632
Right gracilis.....	9:50 a.m.	0.619
Left gracilis.....	10:00 a.m.	0.611
Right gracilis.....	10:31 a.m.	0.572
Left gracilis.....	10:54 a.m.	0.569
Left gracilis.....	12:24 p.m.	0.385

When 0.008 mgm. of epinephrine for each kilogram of body weight was injected intravenously into a dog under iso-amylethyl barbiturate it was found that the average decrease in the glycogen in the four sets of muscles after an interval of one hour was 33 per cent.

Effect of continuous injection of 0.00016 mgm. of epinephrine for each kilogram of body weight for each minute. Three experiments were performed using 0.00016 mgm. of epinephrine for each kilogram of body weight each minute, and the results were found to be consistent. This was the smallest

dosage of the drug which was found to produce a distinct decrease in the glycogen content of skeletal muscle. A representative experiment follows:

The dog weighed 19 kgm. From 8:35 a.m. until 9:00 a.m. 47 mgm. of iso-amylethyl barbiturate for each kilogram of body weight were injected intravenously. During the period from 9:40 a.m. until 11:25 a.m. the animal received 0.00016 mgm. of epinephrine for each kilogram of body weight each minute. The results follow:

MUSCLE	TIME	GLYCOGEN
		<i>per cent</i>
Right sartorius.....	9:05 a.m.	0.526
Left sartorius.....	10:25 a.m.	0.392
Right sartorius.....	11:16 a.m.	0.375
Left sartorius.....	12:10 p.m.	0.318
Right quadriceps.....	9:07 a.m.	0.512
Left quadriceps.....	10:27 a.m.	0.384
Right quadriceps.....	11:17 a.m.	0.336
Left quadriceps.....	12:11 p.m.	0.297
Right gracilis.....	9:08 a.m.	0.309
Left gracilis.....	10:29 a.m.	0.256
Right gracilis.....	11:19 a.m.	0.234
Left gracilis.....	12:13 p.m.	0.234
Right adductor.....	9:10 a.m.	0.501
Left adductor.....	10:31 a.m.	0.334
Right adductor.....	11:20 a.m.	0.289

It is conceivable that the diminution of glycogen in muscles in the foregoing experiments was due to reduction in the blood supply of the muscles due to vasoconstriction. Therefore it seemed expedient to determine whether such a dose of epinephrine causes any change in arterial blood pressure or in volume of blood in a limb. Consequently studies were carried out on two dogs under iso-amylethyl barbiturate, in which arterial blood pressure tracings were done. In one of the animals simultaneous plethysmographic records were made, injecting 0.00016 mgm. of epinephrine for varying periods of time. In no case did this dose cause an elevation of arterial blood pressure or a diminution of limb volume. A final series of experiments was done on the effect of epinephrine on glycogen of muscles, using the dose of 0.000066 mgm. for each kilogram of body weight each minute. It was found that this dose had no effect on the glycogen content of the muscles of the animal injected.

COMMENT. It seems evident from the data presented that large doses of epinephrine have a definite glycogenolytic action, and that doses as small

as 0.00016 mgm. for each kilogram of body weight each minute also exert a glycogen depleting effect. The latter dose is considered to be within physiologic limits, as evidenced by the work of Cannon and Rapport and of Boothby.

The mechanism of the action of epinephrine remains obscure, but it would appear from the data presented that epinephrine does not promote glycogenolysis by effecting a reduction in the caliber of the vessels, particularly in those experiments in which minimal doses were used.

SUMMARY

Iso-amylethyl barbiturate was found to cause little, if any, alteration in the glycogen content of skeletal muscle.

Massive doses of epinephrine caused a marked decrease of glycogen in muscles. Doses of the substance as small as 0.00016 mgm. for each kilogram for each minute caused definite glycogenolysis, whereas 0.000066 mgm. for each kilogram of body weight each minute did not result in demonstrable alteration in glycogen. The former dose is not attended by elevation of the arterial blood pressure or diminution of limb volume.

BIBLIOGRAPHY

- AGADSHANIAN, Z. 1907. *Biochem. Zeitschr.*, ii, 148.
- BEST, C. H., J. P. HOET AND H. P. MARKS. 1926. *Proc. Roy. Soc. London.*, c, 32.
- BLATHERWICK, N. R. AND M. SAHYUN. 1929. *Journ. Biol. Chem.*, lxxx, 123.
- BOOTHBY, W. M. AND I. SANDIFORD. 1923. *This Journal*, lxvi, 92.
- CANNON, W. B. AND D. RAPPORT. 1921. *This Journal*, lviii, 308.
- CORI, C. F. AND G. T. CORI. 1928. *Journ. Biol. Chem.*, lxxix, 309, 321, 343.
1929. *Journ. Biol. Chem.*, lxxxiv, 683.
- CORI, G. T. 1930. *This Journal*, xciv, 557.
- EADIE, G. S. 1929. *This Journal*, lxxxix, 46.
1930. *This Journal*, xciv, 69.
- FÍROR, W. M. AND G. S. EADIE. 1930. *This Journal*, xciv, 615.
- GEIGER, E. AND E. SCHMIDT. 1928. *Arch. f. exper. Path. u. Pharm.*, cxxxiv, 173.
1929. *Ibid.*, cxliii, 321.
- HOUSSAY, B.-A. AND P. MAZZOCCO. 1927. *Compt. rend. Soc. de biol.*, xlvii, 1252.
- MAJOR, S. G. AND J. L. BOLLMAN. 1932. *Proc. Soc. Exper. Biol. and Med.* (In press.)
- MARKOWITZ, J., F. C. MANN AND J. L. BOLLMAN. 1929. *This Journal*, lxxxvii, 566.
- MARKOWITZ, J. AND S. SOSKIN. 1927. *Proc. Soc. Exper. Biol. and Med.*, xxv, 7.
- OHARA, T. 1925. *Tohoku Journ. Exper. Med.*, vi, 1, 23.
- POLLAK, L. 1909. *Arch. exper. Path. u. Pharm.*, lxi, 149.
- RINGER, A. I., H. DUBIN AND F. H. FRANKEL. 1921. *Proc. Soc. Exper. Biol. and Med.*, xix, 92.
- SACKS, J. 1931. *This Journal*, xcvi, 467.
- SAHYUN, M. AND J. M. LUCK. 1929. *Journ. Biol. Chem.*, lxxxv, 1.
- SOSKIN, S. 1927. *This Journal*, lxxxiii, 162.

INHIBITION OF LACTIC ACID FORMATION IN BRAIN AND KIDNEY TISSUE PRODUCED BY INTRAVENOUS INJECTION OF SODIUM MONOiodoacetate

JOHN HALDI

From the Department of Physiology, University of Michigan, Ann Arbor, Michigan

Received for publication May 16, 1932

In a previous paper (Haldi, 1932) an hypothetical explanation was offered for the characteristic different rates of lactic acid formation in the various tissues. It was suggested that these differences might be due to the presence in different concentrations in the tissues of one or more enzymes controlling lactic acid production. The present research puts this hypothesis to the test by carrying a step further the interesting studies of Lundsgaard (1930a) on the effect produced by sodium monoiodoacetate on lactic acid formation in the tissues. Lundsgaard observed that when monoiodoacetic acid neutralized with sodium carbonate was injected into frogs and guinea pigs there was no lactic acid formed either in contracting or excised muscle. He also found (1930b) that in moderate concentrations sodium monoiodoacetate inhibited the enzymatic hexose cleavage that occurs in alcoholic fermentation. On the other hand it had no effect on glycogenolysis, phosphagen cleavage nor on the enzymatic activities of invertase, ptyalin and catalase. Sodium monoiodoacetate therefore apparently has an inhibiting effect on specific enzymes. If the assumption is correct that the same enzymes control lactic acid production in the various tissues, then monoiodoacetic acid should inhibit lactic acid formation in brain and kidney as well as in muscle. Should it be proved that this is not the case it would be necessary to abandon, or at least materially modify the hypothesis attributing the characteristic rates of lactic acid formation in the various tissues to different concentrations of enzymes.

METHODS AND RESULTS. Small dogs of 3 to 6 kilos were used in all the experiments. The animal was decapitated with a T-shaped guillotine, the brain and kidney excised and lactic acid analyses made in the manner described elsewhere (Haldi, 1932).

Since the animals used for studying the effects of sodium monoiodoacetate were anesthetized with morphine and urethane, nine experiments were performed as controls to establish the initial lactic acid content of the brain and kidney of anesthetized dogs and also the accumulation of lactic acid after ten minutes' incubation of the organs. Each animal was injected subcu-

taneously with one gram urethane and 0.4 cc. of a 2 per cent morphine solution per kilo body weight two to four hours before it was decapitated. The initial lactic acid content of the brain in eight out of nine control experiments varied between 50.1 and 60.4 mgm. per 100 grams of tissue. In one experiment it was slightly lower at 45.8 mgm. The average for the nine experiments gave an initial lactic acid content in brain tissue of 54 mgm. per cent 13 seconds after decapitation. In the kidney the initial lactic acid content was 14.5 to 35.1 mgm. per cent with an average of 22.9 mgm. per cent. The average time interval between decapitation and immersion of the kidney in liquid air was 17 seconds. The amount of increase in lactic acid was determined by taking the difference between the percentage content of the second portion of the brain incubated for ten minutes and

TABLE 1

Lactic acid content in milligrams per cent of brain and kidney after intravenous injection of sodium monoiodoacetate

EXPERIMENT	LACTIC ACID (MGM. PER CENT) OF BRAIN DROPPED IN LIQUID AIR 7 TO 42 SECONDS AFTER DECAPITATION	LACTIC ACID (MGM. PER CENT) OF BRAIN INCUBATED 10 MINUTES	LACTIC ACID (MGM. PER CENT) OF KIDNEY DROPPED IN LIQUID AIR 12 TO 59 SECONDS AFTER DECAPITATION	LACTIC ACID (MGM. PER CENT) OF KIDNEY INCUBATED 10 MINUTES	MILLIGRAMS MONOIODOACETIC ACID PER KILO BODY WEIGHT
10	62.0	146.9	15.6	15.6	65.8
11	45.8	141.0	19.8	16.0	68.9
12	43.2	107.8	22.7	15.9	42.8
13	41.3	94.7	20.5	20.6	82.1
14*	41.1	52.0	29.2	25.4	78.5
15	36.2	42.8	26.5	24.5	126.0
16*	31.5	33.9	12.4	10.6	72.2
17*	41.2	41.1	41.4	36.6	49.0

* Animal's heart had stopped beating approximately 1 to 2 minutes before decapitation.

that of the first portion frozen in liquid air immediately after decapitation of the animal. The increase of lactic acid in the anesthetized brain in ten minutes ranged from 89.9 to 142.8 mgm. per cent with an average of 113.3 mgm. per cent. The absolute amount of lactic acid in the brain at the end of approximately ten minutes varied between 140.9 and 192.9 mgm. per cent. In the kidney the increase of lactic acid at the end of ten minutes determined the same way as for brain tissue ranged from 25.2 to 53.7 mgm. per cent with an average of 36.3 mgm. per cent. The absolute amount of lactic acid in the kidney of anesthetized dogs at the end of approximately ten minutes' incubation of the organ was 45.8 to 80.6 mgm. per cent, the average for nine experiments 59.3 mgm. per cent. From these control experiments it was therefore clearly established that excised brain and

kidney from dogs anesthetized with morphine and urethane show a marked increase in lactic acid after 10 minutes' incubation of the tissues.

The results obtained after injection of sodium monoiodoacetate are given in the table. Monoiodoacetic acid was neutralized with sodium carbonate and the solution injected slowly into the femoral vein. It was found in conformity with Lundsgaard's observations (1930) that sodium monoiodoacetate is highly toxic. Susceptibility to toxicity however varies markedly in different animals. In experiment 14 the injection was begun at 10:30 a.m. and continued slowly for approximately twenty-five minutes. At 10:58 the animal was dead, having received 78.5 mgm. monoiodoacetic acid per kilo body weight. In striking contrast, the animal in experiment 15 received 126 mgm. per kilo body weight and was still alive when brought to the guillotine 45 minutes from the time the injection was begun. In this experiment as in the previous one the solution was injected slowly. In experiments 10, 11, 12, 13 and 15 the animal was alive although in poor condition immediately prior to decapitation. The animal was dead in experiments 14, 16, and 17. In these latter experiments one to two minutes elapsed between cessation of the heart beat and removal of the brain and kidney.

A marked difference is observed in the effects produced in the brain and kidney. In no instance was there any formation of lactic acid in the kidney at the end of ten minutes' incubation. On the other hand, in every experiment except 10 and 13 the lactic acid content of the kidney decreased after incubation. In the case of the brain however experiments 10, 11, 12, and 13 show a fairly large increase in lactic acid whereas in experiments 14, 15 and 16 the increase was insignificant and in experiment 17 there was no increase. In experiments 10 and 11 the absolute amount of lactic acid in the brain and the amount formed in ten minutes fall within the limits of the values obtained in the control experiments. In experiments 12 and 13 lactic acid increased during incubation but the amount formed was much less than in the control experiments. Although the concentration of sodium monoiodoacetate was not sufficient to prevent completely the formation of lactic acid, it nevertheless had a marked inhibitory effect. In experiments 14, 15 and 16, sodium monoiodoacetate produced almost complete inhibition of lactic acid. In these experiments lactic acid which normally increased 80 to 100 mgm. per cent in ten minutes increased only 2.4 to 10.9 mgm. per cent. In contrast with the results of the experiments on the kidney the final lactic acid values of the brain were in no instance lower than the initial values. It is interesting to note that there was a complete inhibition of lactic acid formation in the excised brain in only one experiment. In this experiment the dosage was sufficient to kill the animal.

DISCUSSION. Sodium monoiodoacetate in sufficient concentration in-

hibited lactic acid formation in both brain and kidney tissues. Assuming a uniform distribution in the tissues of the injected sodium monoiodoacetate, an amount large enough to inhibit lactic acid formation in the kidney did not always suffice to produce the same effect in the brain. This is what might be expected in accordance with the hypothesis attributing differences in the rate of lactic acid formation in the various tissues to differences in the concentration of one or more enzymes controlling lactic acid formation. A greater amount of sodium monoiodoacetate should be required to counteract the greater amount of enzyme in the brain. The observations, therefore, while they do not offer final proof, nevertheless lend support to the supposition of an enzymatic control of lactic acid formation in the various tissues. In this connection it is of interest to note that Dudley (1931) has reported inactivation of glyoxalase by sodium monoiodoacetate.

The average loss in eight experiments of 2.9 mgm. per cent lactic acid in the kidney raises an interesting question. Can it be accounted for as an oxidation by the oxygen contained in the blood trapped in the excised organ? A similar loss of lactic acid in excised muscle was observed by Lundsgaard (1930a). Theoretically, one milligram of lactic acid should be oxidized by 746 c.mm. of oxygen or 1.34 mgm. by 1 cc. of oxygen. Meyerhof and Himwich (cited by Himwich, Koskoff and Nahum, 1930) found experimentally that 1 cc. of oxygen caused the disappearance of 1.6 to 2.7 mgm. lactic acid in the excised muscle of the rat. Apparently some of the lactic acid was converted into the precursor state. Applying these experimental deductions on the removal of lactic acid in muscle to kidney tissue a maximum of 1.8 cc. or a minimum of 1.07 cc. oxygen would have been required for the removal of 2.9 mgm. lactic acid per 100 grams of kidney tissue. On this basis, assuming that the blood in the excised tissues contained 16 to 18 volumes per cent oxygen, the oxidation would have been effected by a maximum of approximately 10.6 or a minimum of 1.6 volumes per cent of blood. It is possible that the excised kidney contained sufficient blood, in accordance with the above calculations, to account for the observed loss of lactic acid. The actual amount of blood in the tissue, however, was not ascertained.

SUMMARY

Intravenous injections of monoiodoacetic acid neutralized with sodium carbonate inhibited lactic acid formation in excised brain and kidney of dogs anesthetized with morphine and urethane.

In a series of control experiments with no injection of monoiodoacetic acid lactic acid in the brain of dogs showed an increase of 89.9 to 142.8 mgm. per cent with an average of 113.3 mgm. per cent after ten minutes'

worth of the unruptured ripe follicles as criteria of changes in the concentration of gonad-stimulating substances in the blood stream.

METHODS AND MATERIALS. As in our previous experiments post partum female rabbits were used exclusively. The extracts employed were prepared from pregnancy urine by Parke, Davis & Co., and were supplied in sterile bottles of 10 cc. capacity. These extracts were repeatedly subjected to bioassay by the rabbit method (Friedman, 1932b), so that the strength of each extract was known at the time of each intrafollicular injection. The material was kept in the ice chamber at all times except when in use. The technique of the intrafollicular injections was the same as that employed in the earlier experiments (Friedman, 1932a). In the later experiments, however, the volume of the extract discharged from the syringe was measured at the time of operation.

RESULTS. Series I. Group A. An examination of the influence of the contralateral, untreated ovary. In view of the established antagonism between the secretions of ripe follicles and corpora lutea (Leonard, Hisaw, and Fevold, 1932; Hisaw and Leonard, 1930; Courier, 1930a, b; Reynolds, 1932) there existed the possibility that secretions from the ripe follicles of the contralateral, untreated ovary interfered peripherally with the secretions of the unilateral corpora lutea. Moreover, it seemed possible that the structures in the untreated ovary jeopardised the optimal growth of the unilateral corpora lutea by competing with the latter for some essential element in the blood stream. Because of these considerations it was decided to remove the untreated ovary 24 hours after the intrafollicular injection.

Accordingly, all of the ripe follicles in one ovary of each of twenty rabbits were injected with a small quantity of an extract of urine of pregnancy. On the following day the untreated ovary was extirpated and examined for the presence of freshly ruptured follicles. Each of these rabbits was then subjected to a third operation two to five days later (on the third to sixth day after the intrafollicular injection), at which time several linen threads were drawn through the uterus. At autopsy six days afterwards the remaining ovary and uterus were carefully examined internally, and then immediately placed in formalin for subsequent histological examination.

A period of nine weeks elapsed between the first and last of these twenty experiments. Repeated bioassays of the extract employed showed a gradual deterioration from 65 rat units per cubic centimeter to 50 rat units per cubic centimeter during the course of these experiments. The amount of extract discharged from the syringe in the execution of the intrafollicular injections in any one animal was usually between 0.02 and 0.03 cc. and in only one case did the amount exceed 0.04 cc. In not one of the twenty rabbits did the intrafollicular injection result in ovulation from the

untreated ovary. (Minimal intravenous ovulating dose of this extract was from 0.05 to 0.09 cc. per rabbit, depending upon the weight of the animal.)

In nineteen of these twenty experiments the intrafollicular injection was successful, the injected ovary presenting from one to four corpora lutea when examined at autopsy. In seventeen of these successful experiments the nodular swellings found at the site of the thread in the uterus proved to be merely nodules of edematous uterine mucosa without a trace of decidual tissue. Yet, microscopic examination of the tumours in the remaining two of the nineteen rabbits revealed genuine decidual tissue. Grossly, the corpora lutea in these two rabbits were as large and as vascular as the ordinary corpora lutea of pseudopregnancy, and microscopically too, they were indistinguishable from the latter.

It is true that the corpora lutea in these two animals were among the best in this series, and that on the whole the corpora lutea produced in the present experiments were distinctly better than those produced in the earlier work (Friedman, 1932a) where the untreated ovary was not removed. Nevertheless, if one would examine the gross and microscopic appearance of the corpora lutea produced in the nineteen animals of the present series, he would find no good correlation between the character of the corpora lutea and the reaction of the uterus to the thread. In nine animals in which no decidual tumors were formed, the corpora lutea were at least as large, and as vascular, and as sound microscopically as the lutein bodies in the two animals in which deciduomata appeared. Nor was there any correlation between the number of good corpora lutea produced and the formation of a decidual tumor. In the two instances in which the corpora lutea proved to be functional there were three corpora lutea in one animal, and four in the other. In the nine instances wherein the corpora lutea appeared to be quite as good as the functional corpora lutea, there were four animals with two corpora lutea; four, with three good corpora lutea; and one animal in which four good corpora lutea were produced.

Finally, there appeared to be no correlation between the strength of the extract at the time of the irrigation and the functional activity of the resulting corpora lutea, insofar as the two experiments in which functional corpora lutea were produced were in the middle of the series. Nor did the quantity of extract used for the injection appear to make any difference; the quantity used for the production of the functional unilateral corpora lutea being between 0.02 and 0.03 cc. in each case.

B. The regularity of the decidual reaction in the presence of normal corpora lutea. It was assumed at the start of these experiments that decidual tumors could be produced regularly in the presence of functional lutein tissue. The apparent lack of correlation between the structure and the functional activity of the corpora lutea produced in the preceding experiments raised some doubt as to the adequacy of my particular technique for

the production of deciduomata, and made it imperative to put this technique to a satisfactory trial.

Consequently, thirteen female rabbits were given a single intravenous injection of an active extract. Ovulation was verified in each rabbit by inspection at laparotomy on the following day. It so happened that in two of these thirteen rabbits all the corpora lutea save one were in one ovary. In each of these two rabbits the ovary containing most of the corpora lutea was removed, leaving behind only one corpus luteum. Four days later the uterus in each one of these rabbits was slit open along the free border and three linen sutures passed through the muscular layer and mucosa, and tied loosely in place. The technique was exactly that executed in the 20 rabbits of the preceding experiments (A). At autopsy, six days afterwards, nodular enlargements were found at the site of the thread in each uterus. Microscopically the nodules proved to be genuine deciduomata in eleven of the thirteen rabbits. In the remaining two rabbits the nodules consisted entirely of edematous mucosa. It was in these two rabbits, however, that only one corpus luteum was left in place. From gross inspection, the single corpus luteum in each of these two animals was as large and vascular as the corpora lutea in eleven other animals of this group, and was quite comparable to the corpora lutea found at a similar stage of pseudopregnancy resulting from a sterile coitus.

In view of Corner's results with ovarian extirpation during early pregnancy (1928) one might have expected that one corpus luteum would be sufficient to sensitize the uterus for the production of decidual tumors. It is, of course, possible that it was mere coincidence that the only two failures in this series of experiments occurred in the animals in which there was only one corpus luteum. It is more probable, however, that more of the lutein hormone is required for uterine sensitization than is required for the proliferative change. At any rate, it appears evident that the many unsuccessful attempts to provoke the decidual reaction in the experiments of group A cannot be charged to the threading technique or to an irregularity of the decidual reaction.

C. The influence of the operative procedures on corpora lutea produced by intrafollicular injection. After the results in the first two groups of experiments were examined, the factor of operative trauma was more seriously considered as an important element in causing the failure of functional manifestation on the part of most of the corpora lutea produced by intrafollicular injection. In order to evaluate this factor, the follicles in one ovary of seven rabbits were injected with small quantities of an active extract just as in A. At the end of the operation each rabbit was given intravenously one minimal effective dose of extract (about one rat unit per kilo, or 0.02 cc. per kilo of the extract in question). This dosage will provoke ovulation from ripe follicles, but will produce neither ovulation

nor luteinisation in unripe follicles (Friedman, 1932b). On the following day the contralateral, untreated ovary was removed, and it was noted that in each case ovulation had occurred. Five days later the uterus was threaded in all the females of this group, and six days after this operation the animals were sacrificed. In five of these females the injected ovary contained several corpora lutea. In the remaining two does the injected ovary contained neither corpora lutea, corpora hemorrhagica, nor large follicles. Of the five rabbits in which corpora lutea were found decidual tumors were found in only two of them, despite the fact that the corpora lutea in the three other rabbits appeared, grossly and microscopically, to be equally as good. It must be admitted that there were more corpora lutea in the ovaries associated with decidual tumors (six in one ovary, seven in the other) than in the ovaries associated with no decidual reaction (three, three and four). Nevertheless, when one considers that the intravenous injection of a minimal effective dose of an extract of urine of pregnancy results not only in ovulation, but in the development of functional corpora lutea, it is clear that the data obtained in the above experiments give strong support to either or both of two possibilities; namely, 1, that the operative procedures involved in direct intrafollicular injection prevent the growth and development of lutein tissue in an entirely normal manner, even after the application of a stimulus known to be adequate for the normal development of functional lutein tissue (intravenous injection of an active urine extract), or 2, that these procedures somehow prevent the manifestation of full functional activity of normal lutein tissue.

D. The production of functional corpora lutea unilaterally without the extirpation of the untreated ovary. The revelation that a degree of trauma, insufficient to be detected by gross or microscopic examination, could prevent the full functional activity of corpora lutea made it necessary to re-examine the influence of the untreated ovary. It was still possible that the presence of the untreated ovary was the most important factor concerned, and that the factor of trauma appeared as a significant one only when the untreated ovary, with its large follicles, had been removed. But it was now quite as likely that all the results with intrafollicular injections so far could be adequately explained by unappreciated variations in the degree of operative trauma.

To test this hypothesis twelve rabbits were subjected to unilateral intrafollicular injections of an active extract just as in A, with the exception that the untreated ovary was not removed, but allowed to remain in the animal for the duration of the experiment. In each case the uterus was threaded seven days after the first operation, and autopsy performed six days after that. In these experiments the extract employed assayed 65 rat units per cubic centimeter, and the amount of material discharged from the syringe at any one operation did not exceed 0.03 cc.

In eight of these rabbits from one to four corpora lutea were found in the injected ovary at autopsy; in the remaining four animals the intrafollicular injection proved to be completely unsuccessful. In five of the eight successful experiments the corpora lutea found in the injected ovary were distinctly smaller and less vascular than the corpora lutea produced in most of the experiments in group A where the untreated ovary was removed. In the remaining three instances the corpora lutea were as good as the best produced in group A. In two of these three instances decidual tumors were found at the side of the threads. The number of corpora lutea found in the injected ovary of each of these two animals was four. Decidual tumors were found in the uterus of still another animal, however. In this case also the number of corpora lutea was four, but they were distinctly paler and smaller than the corpora lutea seen in the three animals referred to above.

In not one of the twelve rabbits did the follicles of the untreated ovary show any change, grossly or microscopically. The largest follicles in these ovaries did not exceed the size of the largest follicles in an oestral rabbit (1.4 mm.). Microscopic examination showed the granulosa to be intact, and without a trace of lutein transformation. The theca interna was not thickened. In no case was a blood follicle found.

Briefly, the corpora lutea produced in these experiments were not as uniformly good as the corpora lutea produced in group A. Nevertheless the incidence of functional corpora lutea in this group (3 out of 8 cases) was not lower than in A where the untreated ovary was removed (2 out of 19 cases). It is therefore apparent that the presence or absence of the untreated ovary is not of great significance in the production of functional corpora lutea by intrafollicular injection.

Series II. A. The effect of unilateral intrafollicular injections of saline upon ovulation from the contralateral ovary. It had previously been shown that the mechanical rupture of follicles in one ovary immediately after coitus did not prevent ovulation from the contralateral ovary (Friedman, 1931). Although it seemed unlikely that follicles which were subjected to injection would differ markedly in this respect from follicles which had been pricked open, it was necessary to make certain of this. The results obtained in group C of the first series showed that the unilateral intrafollicular injection of an active urine extract did not prevent ovulation from the contralateral ovary following the intravenous injection of a minimal effective dose of the same extract. In these experiments the total amount of extract received by the animal exceeded the minimal effective dose by whatever amount of the extract was absorbed from the injected follicles, and the small and variable amounts which were permitted to leak into the abdominal cavity of the rabbit by any undetected fault in the gauze blockade around the injected ovary. Although it had been

repeatedly shown that such quantities of extract are far too small to have any observable effect on the untreated ovary, it seemed advisable to extend such experiments by substituting saline for the active extracts in the intrafollicular injection.

Consequently the ripe follicles in one ovary of each of three post partum rabbits were carefully injected with saline immediately after coitus (etherisation started 5 minutes after coitus). The technique of the saline injections was identical with that used for the intrafollicular injections in groups A and D of series I. At laparotomy 24 hours later it was seen that ovulation had occurred from the untreated ovary in each of the three does. The incisions were closed in the usual fashion, and the animals permitted to live for four days longer, at which time they were sacrificed and their ovaries preserved for microscopic study. As might be expected, the corpora lutea in the untreated ovaries were almost solidly filled with lutein tissue, there being practically no central cavity. The corpora lutea in the injected ovaries, however, were not solidly filled with lutein cells, there being in each a central cavity of varying size, filled with blood.

Similar results were obtained in three animals in which an intravenous injection of a minimal effective dose of an extract of pregnancy urine was given immediately after the unilateral intrafollicular injection of saline. In no case did the operative procedures prevent ovulation from the contralateral, untreated ovary.

These experiments clearly show that if injected follicles are able to utilise gonadotropic materials more efficiently than unruptured follicles, the difference is not so great as to prevent the unruptured follicles from responding in a normal manner to the humoral changes occurring during the ten or more hours preceding ovulation, whether the ovulation is induced by coitus or by the intravenous injection of a m. e. d. of an active extract.

B. The utilisation of subminimal quantities of gonadotropic material by injected and by unruptured follicles. Despite the decisive character of the results reported in the preceding section, they did not preclude the possibility that a difference in the utilization of gonadotropic material by ruptured and unruptured follicles might become apparent when the concentration of such material was less than that present in the blood during the ten or more hours before ovulation. It had been demonstrated in earlier experiments that repeated intravenous injections of subminimal doses (less than 1 rat unit per kilo) of an active extract would produce in a post partum rabbit either enlarged cystic follicles (2 to 5 mm. diameter), clear cystic follicles with a very narrow lutein border (two to three cells deep), or partially luteinised corpora hemorrhagica, depending upon the size of each dose and the number of doses administered (Friedman, 1932b). If the follicles ruptured by injection were able to utilise the gonad-stimulating substances more efficiently than unruptured follicles, one might expect

to luteinise ruptured follicles with repeated injections of subminimal doses at a rate which would produce little or no luteinisation in ripe, unruptured follicles.

To test this possibility, the following experiments were performed. The ripe follicles in one ovary of each of eight rabbits were injected with sterile saline. The technique employed was the same as that for all previous intrafollicular injections, the amount of saline discharged from the syringe being less than 0.04 cc. per animal. Immediately following the operation, and once each day on succeeding days these eight females were given intravenous injections of an active extract according to the following schedule: rabbits 1 and 2, 0.3 m. e. d. daily for four days; number 3, 0.4 m. e. d. for four days; rabbits 4 and 5, 0.5 m. e. d. for three days; number 6, 0.7 m. e. d. for two days; and rabbits 7 and 8, 0.7 m. e. d. for three days. All of these eight animals were sacrificed six days after the intrafollicular injection of saline, and their ovaries prepared for microscopic examination. In the untreated ovaries of rabbits 1 and 2, there were several cystic follicles, 3 to 5 mm. in diameter, with no trace of luteinisation. The follicles in the injected ovary of these two rabbits were quite as large and quite as free of lutein tissue, although one of the large follicles showed a little blood in the cavity. In all the large follicles of the untreated ovaries of numbers 3, 4 and 5 there was a narrow lutein border (2 to 3 cells deep). This picture was exactly duplicated in the injected ovaries of these three animals, the lutein border being quite as narrow. In rabbit 6, the degree of luteinisation was about the same as that in numbers 3, 4, and 5, and here again there was no detectable difference between the treated and untreated ovary. In this animal, however, there were three partially luteinised follicles in the injected ovary, whereas the untreated ovary contained only one partially luteinised follicle. The degree of luteinisation found in rabbits 7 and 8 was the greatest seen in this group, the lutein border occupying fully one-third of the diameter of the structure on each side, so that the central cavity was limited to somewhat less than one-third of the diameter. Nevertheless the degree of luteinisation in the untreated follicles was equally as great as that found in the follicles in the injected ovary.

The results of these experiments, therefore, give no indication that follicles ruptured by the procedures employed in direct intrafollicular injection are more easily luteinised by subminimal doses of an active extract than are ripe unruptured follicles.

DISCUSSION. Of all the results obtained, the most surprising were those which indicated the relative insignificance of the influence of ripe follicles on the development and functional activity of the unilateral corpora lutea. If there is any *physiological* antagonism between the hormones of the corpus luteum and of the ripe follicle such antagonism was not apparent in these experiments. Equally unexpected were the indications of the

importance of unappreciated differences in the degree of operative trauma. The operator, of course, appreciated that all operations were not consummated with the same degree of success. In some instances a clumsy hand thrust the needle through the follicular wall into the interstitial tissue. Due to such accident, and to others, there was in some cases an abnormally great amount of follicular hemorrhage. At the end of each operation, the experimenter made an attempt to evaluate the success of each of the procedures involved, and his diagnosis of the operation as a whole was recorded on the animal's history card as "poor," "fair," or, "good." In general, the character of the unilateral corpora lutea so produced tallied well with these notations on the history card. In no case did a "poor" operation produce any unilateral corpora lutea. Yet, not every "good" operation was followed by the growth of "good" corpora lutea, and in one case a "fair" operation resulted in the growth of functional lutein tissue. It is obvious, then, that the significant differences in the degree of operative trauma were not registered by the operator.

Nor was the microscopic examination of more service in detecting these differences. It is appreciated that the histological technique employed might have been inadequate to disclose the significant structural differences, and that a more suitable technique might reveal a satisfactory correlation between structure and function. This, however, seems improbable in view of the fact that some of the fully functional corpora lutea were obviously inferior (smaller, paler, cells more vacuolated) to some which were apparently without function. It must be remembered that the ovaries under consideration were removed for study at a time (12th to 14th day) when the corpora lutea of pseudopregnancy have passed the height of their functional activity. There is little doubt that the rate of regression is not identical for all corpora lutea. The chance exists, therefore, that an adequate histological examination at the height of functional activity (7th or 8th day of pseudopregnancy) would regularly distinguish the fully functional from the submaxillary functional corpora lutea.

The phrase "fully functional" is used advisedly here, because of the negative uterine response to the thread in the presence of only one corpus luteum (see group B of series I). It is not unlikely that the decidual reaction requires more lutein hormone than does the proliferative reaction. It is possible that a more sensitive criterion (e.g., inhibition of uterine motility—Reynolds and Allen, 1932) would reveal most of the unilateral corpora lutea produced by the direct injection to be submaximally functional rather than functionless.

Regardless of the degree of functional activity of most of the unilateral corpora lutea, it is significant that some of them were developed and maintained in a functional state in the absence of any humoral changes of

sufficient magnitude to be registered by the untreated follicles of the opposite ovary. If one could assume that these untreated follicles were as sensitive indicators as the ripe follicles in ovaries of an untreated post-partum rabbit, then one could safely say that the growth and maintenance of the functional unilateral corpora lutea in these experiments was effected without the secretion of a special luteinising hormone, and without the continued secretion of a *single* gonad-stimulating factor at a greatly increased rate (certainly less than the amount sufficient to duplicate the humoral condition produced by the daily injection of 0.3 m.e.d. of a urine extract).

Such statement could not be accepted, however, until it could be demonstrated that the sensitivity of the ripe follicles of the untreated ovary was unaltered either by the operative procedures involved in the intrafollicular injections, or by the growth of functional lutein tissue. From the experiments in series II it is readily apparent that the unilateral intrafollicular injection of either saline or an active extract does not prevent the normal response of the ripe follicles in the untreated ovary to those humoral changes which occur in the ten or more hours preceding ovulation. These experiments also show that the partial luteinisation of such ruptured follicles does not interfere with the luteinisation, *pari passu*, of the unruptured follicles in the contralateral ovary. But these experiments do not show whether or not fully functional corpora lutea render ripe follicles less responsive to gonadotropic substances. It has long been accepted that one of the functions of the corpus luteum is the inhibition of ovulation. Even granting this, the question arises as to how this "inhibition" is effected. Is it by decreasing the sensitivity of the ovarian elements to the pituitary secretions, or by decreasing the secretory activity of the pituitary?

Despite the recent work of Patel (1930) there is at hand no satisfactory, direct, quantitative evidence on these questions, but there is much indirect evidence which is relevant. It is known for example, that in most species ovulation does not occur during pregnancy, pseudo-pregnancy, or lactation; i.e., in the presence of a functional corpus luteum. In view of this, it was generally assumed that the cyclic ovarian changes also ceased, and that the follicular apparatus was suspended in a state of rest until the regression of the corpus luteum. There are enough data in the literature, however, to indicate that quite the reverse is true. According to Loeb (1911) the maturation of follicles continues in the pregnant guinea pig. Swezy and Evans (1930) report that pregnancy in the rat does not interrupt the cyclic maturation of follicles. Although the large follicles, which are developed every four or five days throughout pregnancy, do not ovulate they become luteinised to form corpora lutea of about one-third the size

of those of pregnancy. In the rabbit large follicles appear in the ovaries as early as the sixth day of gestation, and from this time on an increasing number of does will accept coitus (Hammond and Marshall, 1925). Yet, insofar as nothing is known of the blood hormone content in these species during pregnancy, it is impossible to say whether or not the ovarian changes in pregnancy are brought about by larger amounts of gonad-stimulating material than are required to produce the same changes in the non-pregnant animal. Blood studies in the mare (Cole and Hart, 1931) have revealed the presence of gonadotropic material in detectable quantities during a limited period of gestation (40th to 150th days), whereas similar studies on non-pregnant mares throughout the greater part of the oestral cycle have failed to reveal such material. In this limited period of gestation large follicles appear and new corpora lutea are formed. Nevertheless, even these interesting findings in the mare are not sufficient to permit a statement as to the effect of functional lutein tissue on the sensitivity of large follicles to gonad-stimulating material.

More directly applicable to this question are the recent experiments of Snyder and Wislocki (1931). In a series of pregnant rabbits they found that there was a progressive decrease in the amount of extract required to provoke ovulation. Whereas 10 cc. or more were required to produce ovulation on the second day after mating, 2 cc. of the same extract produced ovulation on the seventh day after mating. When one considers that implantation of the rabbit embryos takes place on the eighth day after mating, it is apparent that the minimal ovulating dose of the extract in question was less at the height of activity (8th day) of the corpora lutea than during the formative period of these corpora lutea.

In general, then, it is clear that the presence of functional corpora lutea does not prevent the ovarian response to gonad-stimulating material; and particularly in the rabbit it is difficult to interpret the data at hand as indicating an inhibition of the usual gonadotropic effects by the functional activity of corpora lutea, unless there occurs in the blood of the rabbit an as yet undetected, *progressive* increase in the concentration of gonad-stimulating material in the first week of pregnancy. In view of such considerations, and the data secured in the experiments of series II, the production of unilateral corpora lutea by the direct follicular injection of active extracts seems to afford examples of the production and maintenance of functional corpora lutea in the absence of the secretion of a special luteinising hormone, or the secretion of a single gonadotropic substance at a greatly increased rate. A more definite statement than this cannot justifiably be made at this time, and must await quantitative studies on the amounts of active extract required to ovulate ripe follicles in the presence of functional, unilateral corpora lutea.

SUMMARY

1. By the direct intrafollicular injection of extracts of urine of pregnancy it is possible to produce functional corpora lutea unilaterally.

2. The presence of the contralateral, untreated ovary appears to have little or no influence on the development of functional lutein tissue by this means. Moreover, the presence of the ripe follicles in the untreated ovary does not prevent the manifestation of functional activity by the unilateral corpora lutea. If there exists a *physiological* antagonism between the secretions of ripe follicles and those of corpora lutea, such antagonism was not evident in these experiments.

3. Mechanically ruptured follicles are not more easily luteinised than are ripe, unruptured follicles. The direct injection of the ripe follicles of one ovary does not modify the response of the untreated, ripe follicles of the opposite ovary to those humoral changes which occur in the ten or more hours preceding ovulation, or to those humoral changes produced by the repeated intravenous injection of subminimal doses of active extracts.

4. The production and maintenance of functional corpora lutea unilaterally in the absence of any discernible change in the ripe follicles of the untreated, contralateral ovary, appears to afford examples of the growth and maintenance of functional lutein tissue without the secretion of a special luteinising hormone by the pituitary (or any other gland), or the secretion of a single gonadotropic hormone at a greatly increased rate.

BIBLIOGRAPHY

- COLE, HOWELL AND HART. 1931. *Anat. Rec.*, xlix, 199.
CORNER. 1928. *This Journal*, lxxxvi, 74.
COURRIER. 1930a. *C. R. de Soc. Biol.*, civ, 280.
1930b. *C. R. de Soc. Biol.*, civ, 1178.
FRIEDMAN. 1931. *This Journal*, xcviii, 209.
1932a. *This Journal*, xcix, 332.
1932b. *Journ. Pharm. Exper. Therap.* (in press).
HAMMOND AND MARSHALL. 1925. *Reproduction in the rabbit*. Oliver & Boyd, London.
HISAW AND LEONARD. 1930. *This Journal*, xcii, 547.
LEONARD, HISAW AND FEVOLD. 1932. *This Journal*, c, 11.
LOEB. 1911. *Journ. Morphol.*, xxii, 37.
PATEL. 1930. *Quart. Journ. Exper. Physiol.*, xx, 245.
REYNOLDS. 1931. *This Journal*, xcviii, 230.
REYNOLDS AND ALLEN. 1932. *Proc. Amer. Physiol. Soc.*, *This Journal*, ci, 86.
SNYDER AND WISLOCKI. 1931. *Bull. Johns Hopkins Hosp.*, xlix, 103.
SWEZY AND EVANS. 1930. *Science*, lxx, 46.

CIRCULATORY ADJUSTMENTS TO MODERATE EXERCISE IN NORMAL INDIVIDUALS, WITH PARTICULAR REFERENCE TO THE INTERRELATION BETWEEN THE VELOCITY AND VOLUME OF THE BLOOD FLOW

LAURENCE B. ELLIS

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Massachusetts

Received for publication May 6, 1932

Studies reported on the hemodynamic changes in man during exercise have dealt mainly with changes in the volume flow of blood, in the pulse rate and blood pressure, with the physico-chemical properties of the blood and with alterations in the respiratory functions of the organism. The present study is concerned with the effect of exercise on several measurable factors of the circulatory hemodynamics in normal individuals. In particular, the relation of changes in the blood velocity to alterations in the volume flow of blood were investigated. It has generally been assumed that variations in the velocity of blood flow roughly parallel the changes in the total volume of the blood flow in normal individuals. The blood velocity or the circulation rate may be defined as the shortest time in which a particle of blood or injected material passes from one arbitrarily chosen site in the vascular system to another. This circulatory pathway is usually from one of the superficial veins, most commonly in the arm, through the pulmonary circulation to some arterial or venous location, as in the brachial artery, the carotid sinus, the minute facial vessels or the opposite cubital vein. The total blood flow is, of course, the amount of blood pumped by one of the ventricles of the heart in a given period of time. If the total cross-sectional diameter of the vascular bed remains unchanged, changes in the volume of blood flow will produce parallel changes in the velocity. If, however, the cross sectional area of the peripheral capillary bed is altered these parallel changes will not occur. For instance, if the cross-sectional area of the total pulmonary vascular bed is doubled by the opening up of new capillaries and the engorgement of those already open, then the circulation time will be doubled although the total volume of blood flow is unchanged. Therefore, if the circulation rate and volume of blood flow are simultaneously determined in the same individual and are correlated with changes in the arterial and venous blood pressures, with the respiratory exchange of gases, the vital capacity of the lungs, and the

heart rate, significant information regarding the changes which take place in the pulmonary circulation during exercise may be obtained.

METHODS. The experiments were performed on 5 healthy male subjects, all physicians or medical students ranging in age from 24 to 32 years. None were in a state of "training" at the time of the experiments, although one (J. H.) had been athletic in college, and both he and a second (L. E.) were accustomed to frequent, though intermittent, exercise chiefly in the form of squash racquets or tennis. The exercise was in each case performed on a bicycle ergometer equipped with a Prony brake. An attempt was made to have the work done per minute kept constant for each subject throughout the experiment and to have each subject perform a standard exercise of moderate degree. We were not completely successful in this last endeavor since the heat equivalent of the work done actually varied in the different subjects from 0.666 to 0.965 kgm. Cal. per minute. In no instance did the subjects complain of undue fatigue at the end of the exercise period. The experiments were all performed from one to three hours after the last meal. On each subject the experiments were performed in 2 stages. In the first part of the experiment the cardiac output and the blood velocity measurements were made, both at rest and during exercise. In the second part, the remaining measurements were made on a subsequent day under conditions as nearly identical to those of the first day as possible. The room temperature during all experiments was kept at from 23 to 25 degrees Centigrade.

The cardiac output was determined by the Fick principle, using the technique designed for exercise described by Bock, Dill and Talbott (1928). We believe that this method is the most suitable one at present available for determining the cardiac output *during exercise*, and that although the absolute results may be open to some question, it provides an accurate relative indication of changes in blood flow. Individual dissociation curves for carbon dioxide were not determined, as this seemed unnecessary with the comparatively low grades of exercise employed.

The blood velocity of circulation time was measured by the use of sodium cyanide according to the technique devised by Robb and Weiss (1932b). About 0.05 to 0.13 mgm. per kgm. of body weight of the cyanide in 2 per cent solution was injected into the cubital vein and the appearance of its stimulating action on the respiration timed. This gave a measure of the velocity of blood flow from the cubital vein to the right heart, through the lungs, and to the carotid sinus, where the drug acts by immediately increasing the rate and depth of respiration (Heymans et al., 1931). In an extensive series of experiments the originators of this method have compared it with other methods for determining the circulation rate and have found it to be accurate. The method recommends itself particularly by its extreme simplicity, by the absence of any unpleasant side-effects,

by the very transitory nature of its direct effects and by the fact that it can be repeated frequently at short intervals. It is also entirely objective and the circulation time is registered graphically. In the present study the circulation time was graphically recorded on a kymograph, by means of a time marker, an electric signal depicting the moment of injection, and a pneumographic tracing of the respiration. All determinations of the circulation rate were checked by repetition and the results always checked within a second.

The arterial blood pressure was determined by a mercury sphygmomanom-

TABLE 1

The effect of moderate exercise on the cardiac minute and stroke volume outputs, the heart rate, the circulation rate, and the respiratory exchange in 5 normal individuals

SUBJECT	REMARKS	HEART RATE		CO ₂ TENSION			RESPIRATORY MIN-UTE VOLUME	CO ₂ OUTPUT	CARDIAC OUTPUT PER MINUTE	CARDIAC OUTPUT PER BEAT	OXYGEN CONSUMPTION	RESPIRATORY QUOTIENT	CIRCULATION RATE	HEAT EQUIVALENT OF WORK DONE	EFFICIENCY
				Virtual venous											
		mm. Hg	mm. Hg	mm. Hg	liters	cc. per min-ute	liters	cc.	cc. per min-ute	sec-onds	calo-ries per min-ute	per cent			
J. H....	Sitting	80	14	43.2	35.4	7.8	9.3	284	7.6	95	363	0.77	19		
	Bicycling	114	20	58.2	44.2	14.0	26.7	1,195	19.4	170	1,310	0.91	11	0.873	18.7
M. K....	Sitting	93	18	49.5	41.3	8.2	8.3	283	7.8	82	313	0.90	13		
	Bicycling	140	36	68.2	50.2	18.0	29.9	1,442	22.7	162	1,486	0.97	8.5	0.915	15.8
B. M....	Sitting	98	22	47.6	39.1	8.5	7.2	220	5.9	64	277	0.79	15		
	Bicycling	136	32	62.0	45.0	17.0	22.8	962	15.0	110	1,040	0.92	11.5	0.696	18.5
L. E....	Sitting	88	14	47.8	39.5	8.3	9.4	328	8.6	98	363	0.88	17		
	Bicycling	137	21	64.0	46.7	17.3	28.3	1,287	19.8	146	1,335	0.96	8.5	0.965	20.0
S. W....	Sitting	100	17	51.7	42.2	9.5	8.5	265	6.8	68	311	0.84	17		
	Bicycling	139	24	68.0	50.0	18.0	28.0	1,232	19.3	138	1,274	0.96	10	0.844	17.6

eter. The venous blood pressure was measured by the Eyster (1929) modification of the indirect method of Hooker, the measurement being made over a superficial dorsal vein of the hand, the hand and arm being supported extended at a level a few centimeters below that of the right auricle. The pulse rate was counted at the wrist or over the carotid artery. The vital capacity was obtained by a Collins spirometer.

RESULTS. *Cardiac output, heart rate, total ventilation, respiratory quotient, and oxygen consumption.* The changes which these factors undergo when the subject shifts from a resting sitting position on the bicycle to a state of exercise entailing an oxygen consumption of 1.0 to 1.5 liters per minute

are given in table 1, and some of them are graphically depicted in chart 1. They are in close agreement with those reported and discussed by Bock, Van Caulaert, Dill and their associates (1928). In the present series of experiments the resting pulse rates of the subjects tended to be rather higher than one would expect. This was probably due to the psychic stimulation occasioned by the strangeness of the technical procedures.

The velocity of blood flow (circulation rate). The effect of exercise on the circulation time of the 5 subjects is shown in table 1 and chart 1. The circulation rates at rest varied from 13 to 19 seconds in the different subjects, and these figures are in agreement with the normal values found by Robb and Weiss (1932b), using the same method. It will be seen that in every case the velocity of blood flow increased with exercise (i.e., the circu-

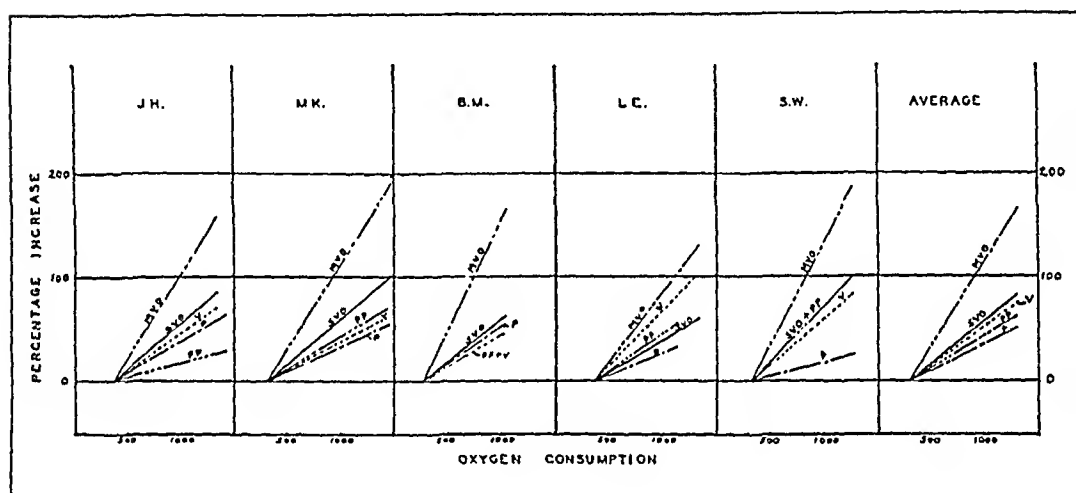


Chart 1. The percentage increase during exercise in the cardiac minute and stroke volume outputs (MVO and SVO), the velocity of the blood flow (V), the arterial pulse pressure (PP), and the pulse rate (P) in 5 normal individuals.

lation time was shortened). This increase in velocity of blood flow was not, however, comparable in degree to the increase in cardiac output, since the velocity increased from 50 to 100 per cent, whereas the volume of blood flow increased from 130 to 190 per cent over the resting value.

The circulation time as determined in the present experiments is essentially a measure of the velocity of venous blood flow from the cubital vein to the right auricle plus the velocity of blood flow through the pulmonary circulation. In normal resting persons with the same method, the circulation time in the vein to the right auricle averages about 5 seconds and the crude pulmonary circulation time is 11 seconds (Robb and Weiss, 1932b). In other words, the venous component of the total determined circulation time is about 30 per cent of the total. In the present experi-

ments it was impossible to determine directly the venous and the pulmonary elements of the velocity measurements during exercise. It is theoretically possible that during exercise such as was employed, which entails muscular activity chiefly in the legs, the velocity of venous blood flow in the comparatively inactive arms is either unchanged or even slightly slower than at rest. If this assumption is made and the results recalculated on this basis then the proportionate increase in the velocity of blood was still less in 4 of the 5 cases than the increase in the cardiac output. However, it is possible to obtain indirect evidence that the velocity of blood flow in the arm during such exercise is actually increased. If a determination of the oxygen content of blood drawn from the cubital vein both at rest and during exercise is made, an increased oxygen saturation of this venous blood during exercise is clear evidence of an increase in the blood flow, since the metabolism of the arm during the exercise is either unchanged or somewhat increased.

This experiment was performed on 5 normal subjects and the results are recorded in table 2. Blood samples were drawn under oil without stasis from the outstretched arm after the subject had remained quietly sitting on the bicycle 10 minutes and again during exercise at the end of 15 minutes of bicycling. The amount of work done was of the same order of magnitude as in the other experiments of this study and the room temperature was maintained close to 25°C. throughout, since it is known (Dill et al., 1931) that variations in the external temperature influence the blood flow in exercise. The oxygen contents and capacities of the blood samples were determined.

In every one of the 5 subjects the degree of oxygen unsaturation during exercise was less than at rest, although the amount of the difference varied in the different individuals. This, then, is substantial evidence in favor of an increased blood flow through the arm during bicycling with the external temperature at about 25 degrees.

Since the size and degree of distention of the peripheral veins during exercise were in no case perceptibly greater than at rest, and in 2 instances (J. M. and A. H.) the veins were clearly smaller, it is safe to assume that the velocity of blood flow in the cubital vein was definitely increased during exercise, although the quantitative extent of this increase could not be definitely computed.

Although it is obviously impossible to draw any definite quantitative conclusions as to the relationship of the increased velocity and total volume of blood flow during moderate exercise, one can say with assurance that both invariably increase in the peripheral and the pulmonary circulations, but in the pulmonary circuit the increase in velocity is of less extent than in the total volume of blood flow.

Arterial blood pressure. (Table 3, chart 1.) The blood pressure of the

5 subjects responded in very different degrees to the exercise, but all showed a considerable rise in systolic and a very slight increase in diastolic pressure, with a consequent increase in the pulse pressure. Similar findings regarding the effect of exercise on the arterial blood pressure have been reported before (Gillespie et al., 1925; Bock, Van Caulaert et al., 1928; Paterson, 1928). The increase in pulse pressure is probably due largely to the increase in the stroke volume output of the heart. The great variability in the blood pressure response of different individuals to exercise, however, is an indication that the adaptive mechanisms in the peripheral circulation are also of great importance in determining the extent of increase in the pulse pressure.

TABLE 2

The oxygen content and capacity of blood drawn from the cubital vein at rest and during exercise

NAME	REMARKS	OXYGEN CAPACITY	OXYGEN CONTENT	PER CENT SATURA- TION	DIFFER- ENCE	HEAT EQUIV- ALENT OF WORK DONE
		<i>volumes per cent</i>	<i>volumes per cent</i>		<i>per cent</i>	<i>calorie per minute</i>
L. E.	{ Sitting Bicycling	20.03	17.80	89	+5	0.848
			18.71	94		
S. W.	{ Sitting Bicycling	20.50	12.11	59	+3	0.847
		21.45	13.33	62		
G. R.	{ Sitting Bicycling	19.58	11.44	58	+7	0.868
		20.19	13.15	65		
J. M.	{ Sitting Bicycling	19.95	10.85	54	+34	0.906
			17.46	88		
A. H.	{ Sitting Bicycling	20.40	6.24	31	+39	0.960
			14.30	70		

Venous blood pressure. (Table 3.) In every case the venous blood pressure rose during the exercise and was either maintained at the increased level until the exercise ended or fell slightly as the work continued. An increase in venous pressure during the performance of exercise has been described before (Hooker, 1911; Schneider et al., 1918; White, 1924; White and Moore, 1925). So far as they go, our findings are essentially in accord with those previously reported. White (1924, 1925) has published results tending to show that during the maintenance of dynamic exercise of a moderate degree of severity there may be a tendency for the venous pressure to fall from its initial high point toward a level that approaches the normal, and with the cessation of the exercise the pressure immediately

becomes normal or subnormal. This finding he ascribes to increased ease of filling of the right side of the heart due probably to a decreased diastolic tone of the heart occurring during the course of the exercise. The increase

TABLE 3

The effect of moderate exercise on the heart rate, the arterial and venous blood pressures, and the vital capacity of the lungs in 5 normal individuals

SUBJECT	REMARKS	TIME FROM START OF EX- ERCISE	HEART RATE	ARTERIAL BLOOD PRESSURE		VENOUS PRES- SURE	VITAL CAPAC- ITY OF LUNGS	HEAT EQUIV- ALENT OF WORK DONE
				Sys- tolic	Dias- tolic			
		minutes		mm. Hg	mm. Hg	cm. H ₂ O	cc.	calorie per minute
J. H.	Sitting	0	60	110	65	6	6,100	0.954
	Bicycling	5	100	140	80	12	5,900	
		10	100	138	78	11	5,950	
	Sitting (5 minutes after stopping)		66	112	70	8	6,200	
M. K.	Sitting	0	82	113	76	3	5,600	0.906
	Bicycling	2	128	144	80			
		7		175	85	5		
		10	126	175	86	3	5,600	
	Sitting (3 minutes after stopping)		96	130	76	3		
B. M.	Sitting	0	72	104	68	4	3,800	0.666
	Bicycling	5	112	130	75		3,750	
		9	116	132	75	8		
	Sitting	0	86	102	78	4	3,850	
	Bicycling	4	130	110	76		3,750	
		10	130	126	80	12	3,800	
	Sitting (2 minutes after stopping)		100	100	76	10		
L. E.	Sitting (12 minutes after stopping)		84			5		0.833
	Sitting	0	84	110	65	1	4,900	
S. W.	Bicycling	5	112			4	4,800	0.895
		10	112	150	80	4	4,800	
	Sitting	0	100	124	82	9	5,100	
	Bicycling	4	126	185	90	13	5,000	
		8	126	180	88	13	5,200	

in venous pressure is evidence of the increased venous backflow to the heart which is produced by the pumping action of the active skeletal musculature and of the diaphragm, by the suction effect of the increased respiratory movements, possibly by a change in the diastolic cardiac tone and, to a

slight extent, by the dilatation of many of the peripheral arterioles and capillaries. The extent to which the pressure rises in any individual case is the resultant of the interaction of these various factors.

Vital capacity of the lungs. (Table 3.) The vital capacity was essentially unchanged during exercise in all the subjects.

DISCUSSION. The changes occurring during exercise, as determined by the various measurements described above, may be correlated by a theoretical analysis of some of the circulatory adjustments which take place in the cardiovascular system. Although the responses of different individuals to a given exercise are qualitatively similar, the quantitative extent of their adjustments is very variable.

With the beginning of exercise the venous backflow to the heart is increased and the venous pressure tends to be raised. Probably as a result of this increased venous return, the heart is stimulated to greater activity and its output is increased.

The fact that during exercise the degree of increase in the velocity of blood flow is consistently less than in the volume of blood flow through the lungs, suggests that during muscular exercise there is, in addition to the increase in the velocity of blood flow through the pulmonary circuit, an increase in the cross-sectional area of the pulmonary vascular pathways. The increase, however, is not marked after such exercise and the main change in the volume of blood flow is due to change in the velocity. Thus the dynamics of the pulmonary circulation during exercise of normal subjects is essentially different from that postulated (Weiss, 1931) and found by Robb and Weiss (1932a) in cardiac patients with circulatory failure at rest. This difference between the behavior of the pulmonary circulation during exercise in normal subjects and in resting patients with circulatory failure is particularly significant as it suggests the possibility that the mechanism of dyspnea in these two states of the body is different. The rôle of the pulmonary circulation and its influence on the physiological state of the alveoli and the lungs as a whole in patients with circulatory failure was discussed, and the mechanism by which an increased pressure in the pulmonary vein, capillaries and arterioles leads to functional emphysema of the lungs was described by these authors. In normal subjects during exercise a similar mechanism in the lungs apparently does not exist.

The experimental evidence that during exercise the cross-sectional diameter of the pulmonary capillary bed increases does not throw light on the question of whether such an increase in the total cross sectional area is due to an increase in the number of open capillaries, or to an increased engorgement of the previously open capillaries. During exercise, however, the blood flow through the visible human capillaries is not, in our experience, associated with engorgement of the individual capillaries and evidence is available that the lung contains reserve capillaries collapsed at rest

(Toyama, 1925; Wearn et al., 1926). The concept that closed capillaries open during exercise appears therefore to be the most probable explanation of the observations reported.

SUMMARY AND CONCLUSIONS

1. In 5 normal individuals estimations were made of the cardiac minute and stroke volume outputs, the respiratory exchange, the velocity of blood flow, the cardiac rate, the arterial and venous blood pressures and the vital capacity of the lungs both at rest and during the performance of a moderate exercise.

2. The velocity of blood flow from the cubital vein through the pulmonary vessels to the carotid artery is increased during exercise to a less extent than the minute volume of the heart.

3. During exercise the systolic arterial blood pressure is definitely increased but there is little or no increase in the diastolic pressure. The venous pressure is increased.

4. The vital capacity of the lungs does not change significantly.

5. The significance of these findings is discussed in relation to the changes in the hemodynamics during exercise.

I take great pleasure in acknowledging my indebtedness to Dr. Soma Weiss for his assistance and advice in this research and to Miss Rose Shore for her technical assistance.

BIBLIOGRAPHY

- BOCK, A. V., C. VAN CAULAERT, D. B. DILL, A. FÖLLING AND L. M. HURXTHAL. 1928. *Journ. Physiol.*, lxvi, 138.
- BOCK, A. V., D. B. DILL AND J. H. TALBOTT. 1928. *Journ. Physiol.*, lxvi, 121.
- DILL, D. B., H. T. EDWARDS, P. S. BAUER AND E. J. LEVENSEN. 1931. *Arbeitsphysiol.*, v, 508.
- EYSTER, J. A. E. 1929. *The clinical aspects of venous pressure*. MacMillan Co., New York.
- GILLESPIE, R. D., C. R. GIBSON, JR. AND D. S. MURRAY. 1925. *Heart*, xii, 1.
- HEYMANS, C., J. J. BOUCKAERT, AND L. DAUTREBANDE. 1931. *Arch. Internat. de Pharmacodyn. et de Therap.*, xl, 54.
- HOOKE, D. R. 1911. *This Journal*, xxviii, 235.
- PATERSON, W. D. 1928. *Journ. Physiol.*, lxvi, 325.
- ROBB, G. P. AND S. WEISS. 1932a. Read by title at the annual meeting of the American Society for Clinical Investigation.
- 1932b. Unpublished observations.
- SCHNEIDER, E. C., G. E. CHELEY AND D. L. SISCO. 1918. *This Journal*, xl, 380.
- TOYAMA, K. 1925. *Zeitschr. f. d. gesamt. exp. Med.*, xlvi, 168.
- WEARN, J. T., J. S. BARR AND W. J. GERMAN. 1926. *Proc. Soc. Exp. Biol. and Med.* xxiv, 114.
- WEISS, S. 1931. *Ann. Int. Med.*, v, 100.
- WHITE, H. L. 1924. *This Journal*, lxix, 410.
- WHITE, H. L. AND R. M. MOORE. 1925. *This Journal*, lxxiii, 636.

FACTORS INFLUENCING ANEMIA DEVELOPMENT IN YOUNG RATS

HELEN S. MITCHELL

From the Nutrition Laboratory, Battle Creek Sanitarium and Battle Creek College

Received for publication May 10, 1932

Nutritional anemia in young rats is produced in most laboratories by feeding a milk ration from the time of weaning or before. In spite of every precaution that the young shall never obtain any of the mother's dry ration, and shall receive only mother's milk and later cow's milk, the hemoglobin content of the blood of young rats at weaning is far from uniform. The variation is but slight among those of the same sex in any one litter but there is a difference between sexes and between litters of the same age and heritage which is not easily explained.

Data accumulated in this laboratory on all rats used for anemia work during the past two years have been analyzed in an effort to determine what factors, if any, are consistently responsible for the variations noted. Complete records of 570 young rats from 73 litters plus blood histories of 42 of the mothers during pregnancy and lactation afford information which should throw some light on the problem. If similar variations have been noted by other investigators, such a study may aid in the standardization of technique to be used in further nutritional anemia work. Details regarding our procedure of bleeding the rats and making hemoglobin determinations are given in a previous paper (1). All hemoglobin figures or averages given in the present paper are based on the average hemoglobin value for all rats of the same sex in any given litter. This procedure avoids unnecessarily detailed work and gives equal weight to the figures from each litter regardless of the number in the litter and the distribution of the sexes.

The differences in the initial hemoglobin figures of the young at weaning seem to be chiefly responsible for differences in the time required to produce a severe anemia. Correlations have, therefore, been attempted between the hemoglobin content of the blood of young rats at weaning and several different factors which might conceivably have some influence on this blood story. These considerations are previous diet of mother, parity of the mother, blood history of the mother during pregnancy and lactation, size of the litter, age and weight of the young at first hemoglobin determination, method of caging and sex differences.

1. *Previous diet of the mother.* The stock ration used in this colony consists of:

	per cent
Dried whole milk.....	20
Rolled oats.....	10
Peanut meal.....	12
Yellow corn (ground).....	10
Dried celery tops.....	3
Dried whole wheat bread.....	42
Wheat germ.....	2
Yeast.....	0.5
Salt.....	0.5

Fresh greens are fed about five times a week. The adequacy of this ration has been demonstrated by eight years' successful use indicated by a high normal growth curve and a splendid reproductive record (2). When the pregnant females are isolated, two or three days before parturition, they are given milk ad libitum and one teaspoonful of wheat germ daily as supplements to the regular stock ration. These and other supplements which have been made in certain cases are always discontinued when the litter is 14 days old to prevent the young obtaining any possible supplementary food. In a previous paper (3) it was reported that neither a mineral supplement in the form of soluble iron, copper and manganese

TABLE 1
Correlation of mother's ration with hemoglobin of young at weaning

	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
Stock ration without supplement.....	53	8.0	8.9
Stock ration and 0.5 mgm. Fe, 0.5 mgm. Cu and 0.1 mgm. Mn daily.....	10	8.8	9.3
Stock ration and yeast extract (Savita) 0.5 mgm. daily.....	7	9.1	10.0

salts, nor an addition of vitamin B complex in the form of a yeast extract exerted any apparent influence in the prevention of the anemia of pregnancy previously noted when animals were fed on the regulation stock ration. In spite of these negative findings it was still possible that the mineral or vitamin supplements to the mothers' ration might increase the resistance of the young to nutritional anemia. Sure and Kik (4) found the concentration of hemoglobin in nursing young to be definitely affected by the diet of the mother. The results of the present survey in this respect are given in table 1.

Although the variation is but slight *the hemoglobin content of the blood of young rats at weaning appears to be influenced to some extent by the previous*

diet of the mother. Experience of other laboratories (5) in which hemoglobin concentrations as low as 2.0 to 3.0 grams per 100 cc. of blood were obtained in rats as early as 8 days after weaning may be due to a stock ration less rich in either the mineral or vitamin B complex than is our original stock ration. No extensive observations have been made in this laboratory on any other type of stock ration.

TABLE 2
Correlation of parity of the mother with hemoglobin of young at weaning

PARITY OF THE MOTHER	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
1st litter.....	17	7.5	8.3
2nd litter.....	20	7.9	8.5
3rd litter.....	12	8.5	9.5
4th-6th litters.....	7	8.9	9.3

TABLE 3
Hemoglobin of young rats at weaning contrasting several successive litters from the same mothers

RAT NUMBER	BIRTH OF LITTER DATE	NUMBER IN LITTER	AGE WHEN SEWE- GATED	HEMOGLOBIN, GRAMS PER 100 CC.		ANEMIA PRODUCED, NUMBER OF WEEKS		RATION OF MOTHER
				Males	Females	Males	Females	
			days					
3339	12/17/29	6	35	6.3(4)	6.3(2)	1.0	1.0	Stock
	2/23/30	9	29	8.1(4)	8.5(5)	4.0	5.0	Stock and Fe, Cu, Mn
	5/ 3/30	11	31	11.1(3)	11.7(4)	5.3	5.2	Stock and Fe, Cu, Mn
	7/27/30	10	33	12.4(6)	12.6(4)	6.0	5.3	Stock and Yeast Extract
3345	9/ 9/29	12	35	5.3(1)	6.4(6)	1.0	1.3	Stock
	11/21/29	11	40	5.4(3)	5.8(4)	1.0	1.0	Stock
	2/ 2/30	12	30	7.3(5)	8.0(6)	4.0	4.0	Stock and Fe, Cu, Mn
	6/17/30	11	28	10.0(6)	11.0(5)	3.5	4.8	Stock and Yeast Extract
	9/ 4/30	5	32	12.3(5)		5.6		Stock

Note: Figures in parentheses indicate the number of each sex used in the experiments.

2. *Parity of the mother.* Litters for anemia experiments have been used as they became available in the breeding colony with no special attention to the parity of the mother. It was noted, however, that first litters seemed to show a more anemic condition at weaning than subsequent litters. A survey from this standpoint has, therefore, been made of all litters used in the present study. The 56 litters in which no dietary change

had been made during the reproductive cycle have been used in arriving at the averages (table 2). These averages afford some evidence that parity of the mother may influence the blood picture of the young but these differences alone would not be significant except for more specific data which happen to be available. In two instances several successive litters from the same mothers were used for anemia work. Hemoglobin values of the young of each litter may, therefore, be compared.

It will be noted that the age when the first hemoglobin determinations were made varies somewhat but there is abundant evidence in our records to indicate that the few days' difference here could not in any way be

TABLE 4

Correlation of hemoglobin level of mother's blood at parturition with that of young at weaning

LOWEST HEMOGLOBIN VALUE OF MOTHER'S BLOOD WITHIN A WEEK OF PARTURITION, GRAMS PER 100 CC. OF BLOOD	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
<i>grams</i>			
15 or above	7	8.5	9.2
13-15	17	8.0	8.7
11-13	15	8.4	9.2
Below 11	3	6.8	6.8

TABLE 5

Correlation of number in litter with hemoglobin of young at weaning

NUMBER OF RATS IN A LITTER	NUMBER OF LITTERS	HEMOGLOBIN, GRAMS PER 100 CC. OF BLOOD	
		Males	Females
4-6	14	8.3	9.0
7-8	24	8.0	8.8
9-10	10	8.3	8.7
11-13	6	7.7	8.4

responsible for the wide differences in hemoglobin figures obtained. To be sure the picture is also complicated by the mineral and vitamin B supplements made to the mother's ration and may to some extent be interpreted in support of the first factor discussed in this paper. However, the *progressive nature of the differences noted in the hemoglobin values for the successive litters from the same mother would seem to be significant*. It is regretted that more extensive data of this type are not available. Other laboratories may be able to contribute further information along this line.

3. *Blood history of mother during pregnancy and lactation.* A study on

anemia in pregnancy recently made in this laboratory (3) affords data on the blood history of the mothers of 42 of the litters used in the present survey. Hemoglobin figures are available at weekly intervals throughout the gestation and lactation periods. The mothers show a significant loss in hemoglobin concentration during gestation with a minimum value just after parturition in most cases, occasionally just before. The 42 litters have been grouped according to the minimum hemoglobin concentration of the mothers' blood within a week of parturition.

There is little, if any, correlation shown in these figures unless the average figure for three litters in the last group may be considered significant. One must conclude that the *hemoglobin concentration of the mother's blood does not show any striking influence on that of the young unless the mother shows a severe degree of anemia*. Such a condition would logically predispose the young to anemia because of an inadequate storage of iron.

4. *Size of the litter*. It is logical to suppose that the more young a mother must nourish either pre- or postnatally the greater the drain on her system and the greater the chances for the young to be malnourished. To apply this theory to the blood story in the present survey all litters (54) fed on stock ration free from complicating dietary influences are grouped according to the number of young in the litter (table 5).

Much to our surprise there *appears to be little, if any, correlation between the number of rats in a litter and the hemoglobin value of their blood at weaning*.

5. *Age and weight of young when first hemoglobin determinations are made*. All conditions of experimental procedure have been kept as uniform as possible throughout the past three years. Rats are from a stock colony which has been practically inbred for 18 generations. Mother rats are always segregated from their litters at 21 days, and first hemoglobin determinations on young scheduled for an anemia experiment are made between the 26th and 36th day at which time the rats are separated into single cages. The body weights vary between 40 and 60 grams at this age. Rats of exactly the same age and weight may show as wide differences in hemoglobin values as those showing the maximum variation in age and weight within the above limits. Thus *no striking correlation seems to exist between the exact age or body weight of the young rats and the hemoglobin content of the blood*. During the first few weeks of rapid growth a normal rat on an adequate ration rapidly brings its hemoglobin up to a normal concentration (3) but on a milk ration there appears to be a period when but slight change occurs followed by a reduction of hemoglobin as the iron reserves are exhausted.

6. *Method of caging*. Several laboratories have recently emphasized the necessity of avoiding mental contamination from cages used for anemic rats. Nevans and Shaw (6) call attention to the material of which the cages are composed and access of the rat to fecal material. In this labora-

tory the cages used for mother rats with young litters have $\frac{1}{4}$ inch mesh which allows some of the feces to remain in the cages. While this source of error is fully recognized it has been a constant factor in all the work

TABLE 6
Comparison of anemia development in males and females

NUMBER OF ANIMALS AND PERIOD OF OBSERVATION	HEMOGLOBIN AVERAGES, GRAMS PER 100 CC. OF BLOOD		AVERAGE NUMBER OF WEEKS ON MILK RATION		NUMBER OF LITTERS IN WHICH AVERAGE HEMOGLOBIN VALUES SHOWN		
	Males	Females	Males	Females	♀ > ♂	♀ = ♂ ± 2%	♀ < ♂
Total 73 litters, 283♂, 291♀: At weaning, average.....	8.2	9.0			58 (80%)	8 (11%)	7 (9%)
Group I. 42 litters, 154♂, 158♀: At weaning, average	7.9	8.5			32 (76%)	6 (14%)	4 (10%)
Range.....	(5.3 -13.1)	(5.9 -13.7)					
End of period on milk, Average	4.1	4.8			33 (79%)	6 (14%)	3 (7%)
Range.....	(3.0 -6.2)	(3.5 -6.7)					
Time constant for all rats in one litter, Average			3.7 (1-12)	3.7 (1-12)			
Range.....							
Group II. 31 litters, 129♂, 133♀: At weaning, average	8.6	9.5			26 (84%)	2 (6%)	3 (10%)
Range.....	(6.3 -12.6)	(6.5 -13.7)					
End of period on milk Average	3.5	3.6					
Range*.....	(3.0 -3.9)	(3.1 -4.0)					
Time required for anemia to develop, average			5.2 (2.0 -8.6)	6.3 (2.7 -13)	26 (84%)	3 (10%)	2 (6%)
Range.....							

* Hemoglobin below 4.0 per 100 cc. for each individual rat.

Note: The hemoglobin values in 10 females of group II failed to decline to the desired level within 10-12 weeks on the milk ration and are, therefore, omitted from the above averages. In no case was a similar delay noted in males.

reported and could scarcely have been responsible for the wide variations in hemoglobin values noted in different litters considering that the values within any one litter are relatively uniform. When the rats are segregated

for nutritional anemia experiments they are placed in individual galvanized wire cages with raised bottoms of $\frac{3}{8}$ inch mesh. All feces drop through this mesh readily.

Since the chief consideration in this discussion thus far has involved the hemoglobin titre in young rats at the time the experiments are started the problem of the individual cages is irrelevant. These cages, however, should enter into consideration in relation to the declining hemoglobin values in rats during the preliminary experimental period on a milk ration. During the latter part of 1931 it was noted that the time required for reduction of hemoglobin values to 4.0 grams per 100 cc. of blood or below required longer than formerly. Some old cages which were in use at that time were suspected of being the source of supplementary minerals. A group of freshly regalvanized cages proved successful in solving this difficulty. In another instance the crowded condition of the laboratory forced us to put a litter of young together in a large cage with $\frac{1}{2}$ inch mesh bottom for two weeks of the preliminary period on milk instead of segregating them in individual cages as was customary. Instead of declining values, rapid hemoglobin synthesis occurred resulting in normal values in two weeks. In this case a rusty metal drinking cup had been used inadvertently in place of the usual glass or porcelain. Faulty caging facilities can easily vitiate all other results, as has been emphasized by other investigators.

7. *Influence of sex.* Our attention was attracted to a sex difference by the hemoglobin records of male and female litter mates which are customarily recorded on one page. Invariably the addition of mineral supplements had to be delayed longer for the females than the males. This phenomenon has proved to be the most interesting of all the factors considered in this survey. The greater resistance to anemia among females is evident no matter how the data are manipulated.

In our earlier work all of the young from one litter remained on the milk ration the same length of time and were given the mineral supplement when some or most of them had developed a severe anemia. The first 42 litters designated as group I, have therefore been studied relative to the comparative hemoglobin titre in males and females in each litter after a constant number of weeks on the milk ration. The second group of 31 litters in which a definite severe degree of anemia was allowed to develop in each rat before any supplement was added regardless of the time involved has been designated as group II. In this group comparison is made of the time required for the hemoglobin values to decline to below 4.0 grams per 100 cc. of blood.

The blood of *female rats* not only shows a higher hemoglobin concentration at weaning but females require longer to develop a severe anemia of a definite degree than do male rats from the same litter. In some instances this

delay seems to be due to a slower rate of decline in hemoglobin in females but from the general averages it would appear that the chief difference is in the initial hemoglobin values. The difference in the growth impulse between the two sexes might also be suggested as a possible factor affecting hemoglobin synthesis. A comparison has, therefore, been made between the rate of gain in weight versus the rate of loss of hemoglobin but there is no direct or inverse correlation apparent. It may, therefore, be concluded either that the female is endowed with a better prenatal storage of iron or that she uses the iron which is available from endogenous or exogenous sources more efficiently than does the male.

SUMMARY

1. An attempt has been made to correlate the variations noted in the hemoglobin content of the blood of young rats at weaning with several factors which might conceivably have some bearing on the problem.

2. Hemoglobin values in young rats at weaning show slight but significant correlation with the ration of the mother during pregnancy and the parity of the mother.

3. No significant correlation is apparent between the hemoglobin content of mother's blood at time of parturition and that of the young at weaning.

4. Neither age and weight of young at weaning nor size of the litter show any apparent influence on blood hemoglobin values at weaning.

5. Metal contamination from cages and food cups used may seriously interfere with the experimental production of nutritional anemia in young rats. Precaution should also be taken to have raised cage bottoms of sufficiently large mesh ($\frac{3}{8}$ - $\frac{1}{2}$ inch) so that the rats do not have access to feces.

6. The sex difference is most interesting because of a consistently higher hemoglobin content of the blood of females than males and a correspondingly longer period required for severe anemia to develop.

BIBLIOGRAPHY

- (1) MITCHELL, H. S. AND MILLER. Bull. B. C. San. and Hosp. Clinic, 1931, xxvi, 225.
- (2) MITCHELL, H. S. Bull. B. C. San and Hosp. Clinic, 1930, xxv, 185.
- (3) MITCHELL, H. S. AND L. MILLER. This Journal, 1931, xcvi, 311.
- (4) SURE, B. AND M. C. KIK. Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 603.
- (5) ELVEHJEM, C. A. AND A. R. KEMMERER. Journ. Biol. Chem., 1931, xciii, 189.
- (6) NEVANS, W. B. AND D. D. SHAW. Science, 1900, lxxii, 249.

STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE

IV. RESPONSE TO AUTONOMIC DRUGS

R. D. TEMPLETON AND HAMPDEN LAWSON¹

From the Physiological Laboratory of the University of Chicago, and the Department of Physiology of Loyola University School of Medicine

Received for publication May 19, 1932

It was found in earlier work (1) that a reciprocal relationship existed between two types of activity observed in the dog's colon. The wave-like contractions characteristic of the proximal colon were found to alternate with rhythmic pulsations, which were usually seen most marked in the anal sphincter region.

Assuming that the innervation of the sphincters is such that the thoracico-lumbar fibers are essentially motor, we were interested in seeing whether, in response to sympathetic drugs such as adrenalin, we could elicit contractions of the sphincter region, along with the rhythmic pulsations throughout the colon usually associated with sphincter activity in spontaneous motility.

It had been observed that rhythmic pulsations of the distal colon could be set up by suitable mechanical or electrical stimulations of the sphincter region (2). In this, as well as in similar activity originating spontaneously, usually the entire distal colon responded as a unit, and occasionally the whole colon.

It was expected, then, that adrenalin would activate the sphincter region, and it was hoped that the response of the rest of the colon might throw some light on the origin of the rhythmic pulsations.

METHODS. In the unanesthetized dog, we used both the six-balloon technique (1) and the plunger-balloon technique for longitudinal and circular activity (3), (4) previously described. The control conditions were exactly similar, with either technique, to those described in previous work. All injections were made intravenously, the crural vein being usually selected. Control injections of physiological salt solutions were given. Adequate time was allowed to elapse (1 to 2 hours) for recovery from the effects of inserting the apparatus.

In anesthetized dogs and cats the plunger-balloon apparatus was used exclusively. The belly was opened by a midline incision from the xiphoid

¹ Donnelley Fellow in Physiology.

process to the symphysis pubis, and the walls were retracted and supported by tying to uprights attached to the animal board, so as to form a deep trough in which the viscera lay exposed. Ringer's solution at body temperature was passed through the open belly by means of a pump so arranged as to re-circulate the fluid, keeping the volume in the abdomen always constant. Temperature of the immersion fluid was kept constant within about $0.2^{\circ}\text{C}.$ by passing the fluid in the pumping system through a series of coils immersed in a thermostatically controlled constant temperature bath before delivery into the abdomen. The total volume of the fluid used in this system was usually about 1500 cc., about one-half of which represents the volume of the pump, tubes, and coils.² Temperature of the fluid in the belly was allowed to vary in different experiments between 39° and $40^{\circ}\text{C}.$ without affecting the results. In about one-fourth of the experiments 0.9 per cent NaCl was used as immersion fluid in place of Ringer's, and one experiment was done in which physiological sucrose solution was substituted. In no case were the results altered.

The plunger apparatus was inserted through the anus and the free end of the plunger stitched in place through the wall of the intact colon, by passing the threaded needle through a perforation in the small metal ball which caps the end. Usually a balloon was also inserted into the proximal colon so as to lie from 3 to 5 cm. above the end of the plunger, through an incision near the cecum. In most cases blood pressure was also recorded by carotid cannulation.

In the dogs, ether anesthesia was used except in 6 experiments in which sodium barbital, about 250 mgm. per kgm. body weight, in divided intravenous doses, was substituted. Ether administration was done by the usual method of tracheal cannulation, and connection to an ether bottle. In the cats, barbital was given intraperitoneally, and supplemented by intravenous doses after the belly was opened. All injections in anesthetized animals were made into the exposed external jugular vein.

In the observations which follow we shall refer to movements of the plunger as longitudinal, or distal longitudinal; to pressure changes in the balloons arranged in series on the plunger apparatus as circular or distal circular; and to pressure changes in the balloons lying free in the proximal segment, in which circular and longitudinal components are not separated, as proximal activity (3).

CONTROL ACTIVITY IN ANESTHETIZED DOGS. Under light or moderate ether anesthesia the colon of the dog shows strong longitudinal activity in the distal segment (fig. 2). Six experiments were done in which the animal was allowed to remain undisturbed except for changes in depth of

² The mechanical details of the circulating system were supplied by Mr. Sidney Smith, Jr.

anesthesia until death. These tracings, which lasted from 6 to 8 hours, show longitudinal activity more or less continuously throughout. The balloons, on the other hand, in both the proximal and the distal segment, are usually quite inactive in such tracings. There is none of the marked periodicity in the longitudinal record which is characteristic of the unanesthetized dog, although the character and rate of the individual contractions on the longitudinal tracing are similar to those of the unanesthetized animal.

When the depth of anesthesia was increased, the longitudinal activity was quickly depressed, until the entire color, both balloon records and longitudinal tracing, showed complete quiescence. Decreasing the depth of anesthesia was followed by return of activity in the longitudinal record within 2 to 5 minutes, and often without any marked change in carotid blood pressure (fig. 1). In those instances in which the balloons were also active under light anesthesia, it was observed that in rate of contractions, periodicity, and tone changes, the balloon lying in the proximal colon corresponded with the plunger rather closely, while the balloons lying below the plunger, in the distal colon, were either completely quiet, or showed negative pressure waves coinciding with longitudinal contractions (fig. 2). In some cases, the balloon lying just below the end of the plunger, (highest in the distal group) showed activity corresponding to that of the plunger and to that of the proximal balloons, while those lying lower in the distal group were either quiet or showed negative waves. Under ordinary anesthesia, there was never any activity in the lower distal balloons of sufficient magnitude to be comparable to that in the unanesthetized dog.

In the dogs which were anesthetized with barbital, there was no gross difference in either longitudinal or circular activity in the distal segment, or balloon activity in the proximal segment, from those in which ether was used (fig. 3). In the work which follows, ether was the anesthetic of choice, as we were able, with it, to obtain control activity of almost any desired magnitude by modification of the depth of anesthesia.

Two cats were used, in which the anesthetic was barbital. Control activity was similar to that in the anesthetized dog. It is not possible, at this time, to make a comparison with the unanesthetized cat.

Adrenalin. Ninety-five injections were made in 20 dogs under ether anesthesia; 21 in 6 dogs under barbital; 65 in 27 experiments on 6 unanesthetized dogs; and 7 in 2 cats under barbital. Doses have varied from 1 cc. of a 1:50,000 solution to 1 cc. of a 1:1000 solution. The usual dose has been 1 cc. of a 1:2000 solution. In this study the synthetic preparations Adrin (H. K. Mulford Co.) and Suprarenin (Metz Laboratories) were used exclusively.

In the dog under moderate anesthesia, with the longitudinal tracing capable of showing either augmentation or depression, and the balloons

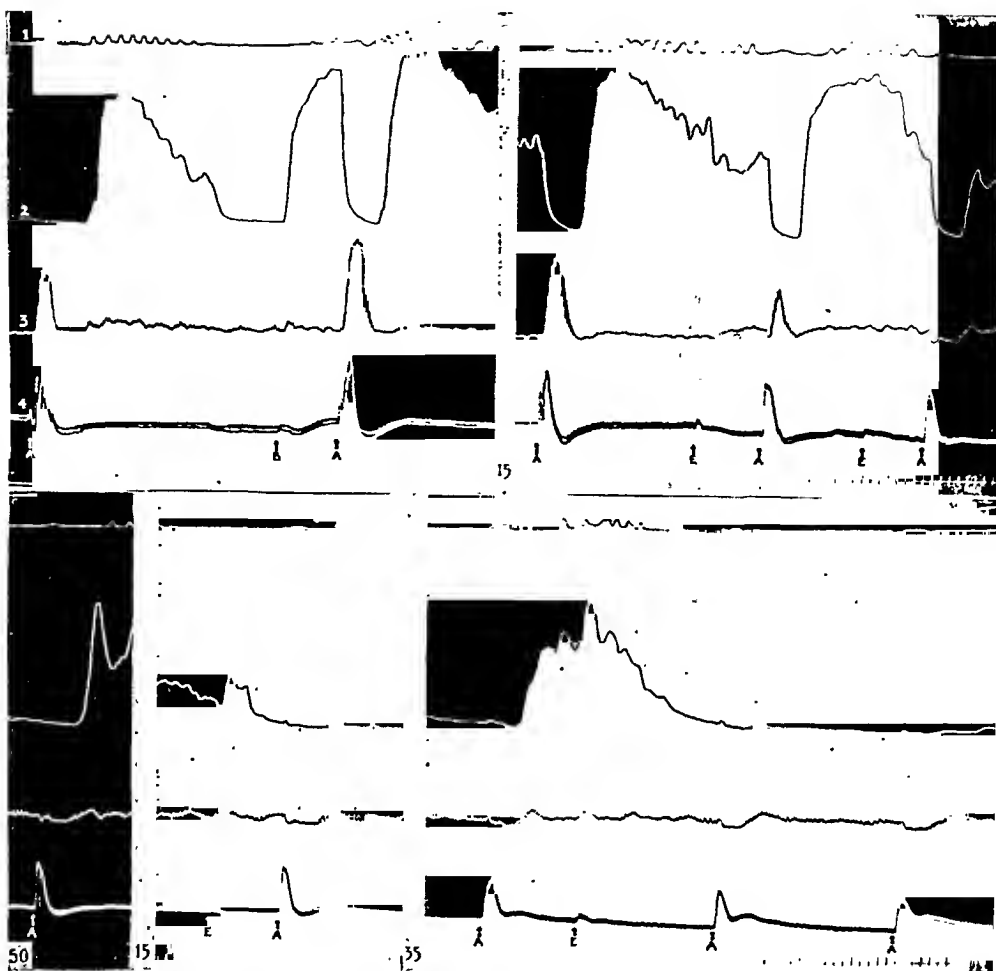


Fig. 1. Dog, 15 kgm. Ether anesthesia. Ringer irrigation at 39°C. 1 is proximal balloon; 2, longitudinal record of distal colon, plunger fastened in place 15 cm. above internal anal sphincter; 3, balloon record of distal colon, 6 cm. above internal anal sphincter; 4, carotid blood pressure. Error in vertical alignment of records less than 5 seconds. Time tracing in minutes. At the points marked *A* 1 cc. adrenalin 1:2,000 was given. At the points marked *E* 1 mgm. ergotamine was given. At *B* the anesthesia was lightened, with almost immediate augmentation of longitudinal tone, following which subsequent adrenalin injections show pronounced preliminary depressant effects on the longitudinal. The first injection of adrenalin was given on prolonged longitudinal depression as a result of deep anesthesia, and shows longitudinal augmentation after a latent period of about 6 minutes. Between the 5th and the 6th adrenalin injections (about 1 hour) the anesthesia was deepened to obtain a depressed longitudinal tone for subsequent injections. After the 2nd ergotamine injection, adrenalin no longer produces augmentation of the distal balloon (no. 3), but still evokes the original longitudinal response. Adrenalin given shortly following the 3rd and 4th ergotamine injections (10 to 15 minutes) fails to elicit longitudinal augmentation, but this response returned when 63 minutes was allowed to elapse between the 3rd ergotamine injection, and the 2nd subsequent adrenalin.



Fig. 2. Dog, 17 kgm. Ether anesthesia. Ringer irrigation at 39°C. 1 is longitudinal record of distal colon, plunger fastened in place 15 cm. above internal anal sphincter. 2, 3, and 4 are serial balloons in the distal colon, 4 just above the internal anal sphincter, 2 highest in the distal segment, about 5 cm. below the plunger. 5 is a proximal balloon, and 6 carotid blood pressure. Time tracing in minutes. Error in vertical alignment of records less than 5 seconds. Control activity before the first injection shows continuous longitudinal activity under moderate anesthesia. The sphincter balloon (no. 4) shows negative waves correlated with positive waves on balloon 2. Balloon 3 is almost quiet. At the points marked A 1 cc. adrenalin 1:2000 was given, at the points marked E 65 mgm. ephedrine, and at the point marked P, 5 mgm. pilocarpine. At B anesthesia was lightened until the palpebral reflex was easily elicited, with resumption of previous anesthetic level after the next following injection. The first adrenalin injection is followed by the typical diphasic response. In the second phase the longitudinal tone is augmented over its active control. The first ephedrine injection produces augmentation in balloons 3 and 4, with depression in all other records. The next two adrenalin doses show the circular response in balloons 2, 3, 4, and 5, but there is no longitudinal augmentation, even in the extremely light anesthesia following B. Pilocarpine produces simultaneous augmentation on all records, most marked on the longitudinal and the proximal balloon. With the next adrenalin injection (4th) 210 minutes after ephedrine, the typical diphasic response is obtained. This is repeated in the 5th. A second ephedrine injection fails to produce maintained elevation of blood pressure, to produce any alteration in motility, or to influence a subsequent adrenalin response.

quiet, the immediate effect of intravenous injection is depression of the longitudinal activity coincident with the blood pressure rise, and coincident with marked augmentation of the balloons (fig. 3). The augmentation of the balloons is most marked in the region of the anal sphincters, but is definite even on the proximal balloons. The effect on the balloons is characteristic, and can be subdivided into two parts (fig. 4), the first of which is a smooth, sudden rise in tone lasting from $\frac{1}{2}$ to 1 minute. The second rise follows after a slight drop in tone, and consists of a high, well-

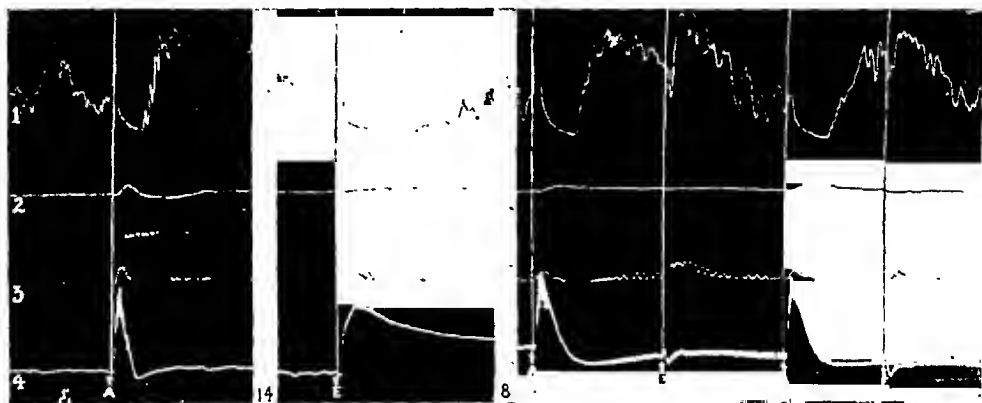


Fig. 3. Dog, 12 kgm. Sodium barbital anesthesia (3 gm.). Ringer irrigation at 39.5°C. 1 is longitudinal record of distal colon, plunger fastened 15 cm. above internal anal sphincter. 2 is balloon in distal segment, 5 cm. above internal anal sphincter. 3 is a proximal balloon, and 4 carotid blood pressure. Error in vertical alignment of records less than 10 seconds. Time tracing in minutes. At the points marked A 1 cc. adrenalin 1:2000 was given, at the points marked E 65 mgm. ephedrine. The first adrenalin injection gives typical diphasic response. Circular augmentation during the first phase is well marked in the proximal balloon. First ephedrine produces longitudinal depression with circular augmentation, with quick recovery. A second adrenalin injection 27 minutes after ephedrine, when motility has returned to control conditions, gives a typical response. Second and third ephedrine injections produce slight depressions in blood pressure, with slight augmentation of longitudinal tone and motility. Adrenalin shortly following (12 minutes) the second ephedrine injection, produces typical diphasic effect.

marked tone change, usually much higher than the first, upon which are usually superimposed well-formed type I contractions. These two effects occupy from 2 to 4 minutes. When the two parts of the effect are easily separated on the balloon records, it can be seen that the first, smooth tone rise is concurrent with the rise in blood pressure, while the second, characterized by the augmented type I contractions, occurs during the return of blood pressure to normal (fig. 2).

Then follows a period of quiet, lasting from 1 to 5 minutes, on the balloons. During this phase, the longitudinal suddenly becomes active,

and invariably is augmented in tone and activity over its control. The augmented longitudinal activity lasts from 4 to 20 minutes, with return to the control level. During the period of augmented longitudinal activity the proximal balloon is usually also augmented, while the balloons below the plunger are irregular in behavior, sometimes being completely depressed, sometimes being augmented. In the latter event, waves of contraction can frequently be traced over the distal set of balloons in a peristaltic direction.

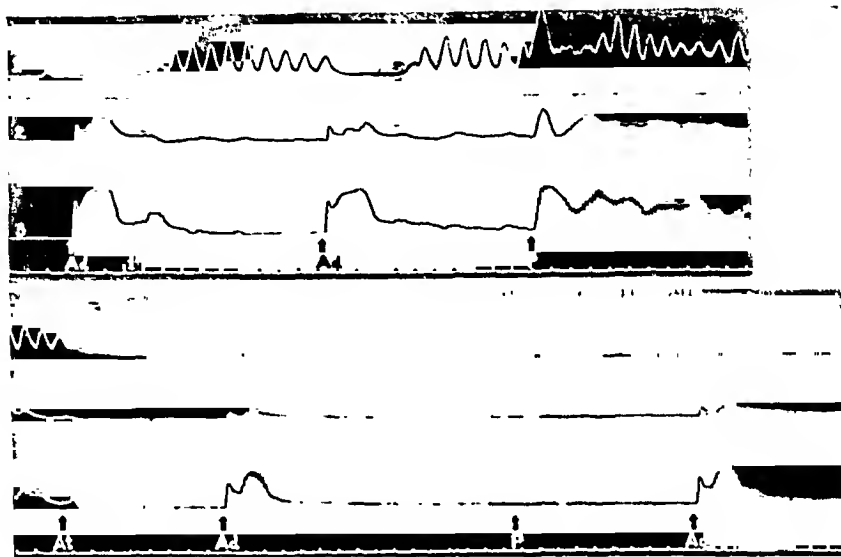


Fig. 4. Dog, 15 kgm. Ether anesthesia. Nine-tenths per cent NaCl immersion fluid. Temperature kept between 38° and 39°C. by large electric lamp. 1 is longitudinal record of distal colon, plunger fastened 15 cm. above internal anal sphincter. 2 and 3 are distal balloons, 3 just above internal sphincter, and 2 3 cm. above. Error in vertical alignment not greater than 15 seconds. Time tracing in minutes. At the points marked *Ad* 1 cc. adrenalin 1:1000 was given, at *P*, 3 mgm. pilocarpine, and at *At*, 3 mgm. atropine. The first two adrenalin injections evoke typical responses. Pilocarpine given during the 2nd phase of the response to the 2nd adrenalin injection produces immediate augmentation on both longitudinal and balloon records. Following atropine adrenalin produces only the first phase of the typical response, longitudinal augmentation being absent. Pilocarpine following atropine fails to augment either circular or longitudinal.

If the depth of anesthesia is increased, so that there is no longitudinal activity appearing on the record, the delayed augmentory effect is more striking (fig. 1). Following the injection, with a latent period of from 3 to 10 minutes, there is a sudden activation of the longitudinal, with a tone rise and contractions lasting as long as 20 minutes. The circular response, and its relationship to the longitudinal and the blood pressure, is the same as in moderate anesthesia. With very light anesthesia, the imme-

diate depressant effect on the longitudinal is made more striking, without altering in any other particular the total response (fig. 1).

These effects were duplicated on cats under barbital, and on dogs under barbital.

On the unanesthetized dog the response was essentially the same, with the exception of some of the time relations. There was almost no latent period between the circular augmentation and the longitudinal augmen-

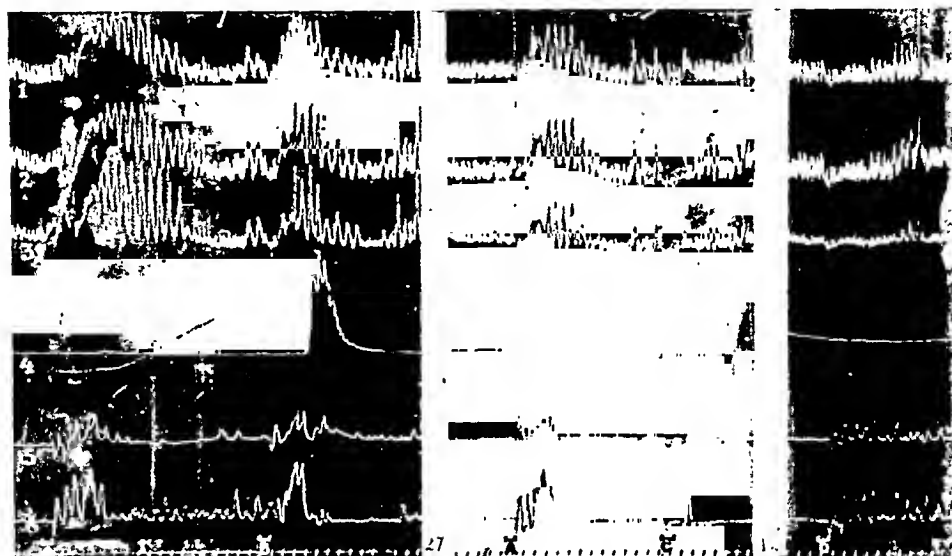


Fig. 5. Dog, 20 kgm. Unanesthetized. Colon transected 15 cm. above left colic vessels, colostomy and distal colon pouch. 1, 2, and 3 are balloons in the proximal segment at intervals of 5 cm. 4 is longitudinal record of the distal pouch, and 5 and 6 are balloons in the distal pouch, 6 just above internal sphincter, and 5, 3 cm. above. Error in vertical alignment less than 5 seconds. Time tracing in minutes. At the points marked A, 5 cc. adrenalin 1:50,000 were given, at B, 10 cc., and at C, 1 cc. of the same. At D, 10 cc. 0.9 per cent NaCl were given in the same manner as all other injections. With the 5 cc. dose, A, the response is typical except that the longitudinal augmentation is lacking during the second phase. With the 10 cc. dose, the complete response is obtained. The 1 cc. dose has no effect. Injection of saline has an effect on the distal balloons totally unlike the effect of adrenalin. (This effect was not constant, many of the animals giving no motility response to the injection of saline controls.)

tation. The division of the immediate circular augmentation into two parts was more difficult, the whole effect on the balloons in this phase, in both proximal and distal segments, lasting about 3 or 4 minutes (figs. 5, 6). Coincident with the termination of the balloon activity, the longitudinal was augmented in both tone and activity. During the augmented phase of the longitudinal, proximal balloons were also augmented, frequently showing peristalsis, and, in other cases, where peristalsis did not

appear, showing high, well-formed, regularly rhythmic type II contractions. During this period, in which the proximal balloons and the distal longitudinal were active, the distal balloons were quiet. Due to the absence of an interval between the immediate (circular augmentory) and delayed (longitudinal augmentory) effects, the proximal balloons usually show, in the unanesthetized dog, continuous augmentation following the injection. Coincident with the longitudinal augmentation and the distal circular depression, however, there is a marked change in character of the

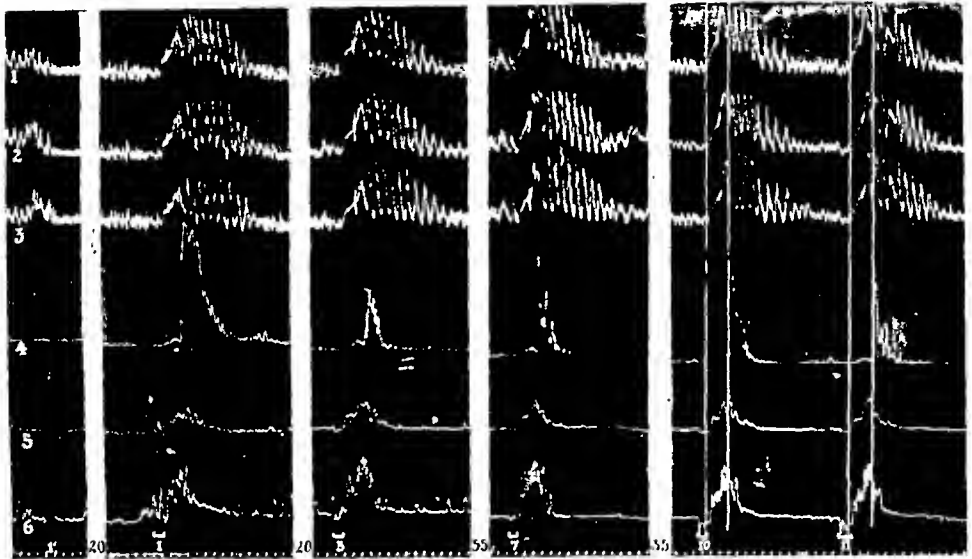


Fig. 6. Preparation and records the same as figure 5. At the points numbered and indicated by bars, 15 cc. adrenalin 1:50,000 were given. These are extreme responses in a series of 11 injections during the course of a single tracing, given at approximately 20 minute intervals. The serial number of the injection is given below the bar marking the injection. The vertical lines drawn through responses to injections 10 and 11 divide the response into the two phases, the first line signalling beginning of the first phase, the second the beginning of the second. The first phase is well marked in the proximal segment. Error in vertical alignment of records not greater than 10 seconds.

proximal activity (fig. 6). During the distal circular phase, the proximal balloons show essentially the same type of activity as the distal circular records, i.e., high type I contractions on an irregular tone. Coincident with the longitudinal augmentation in the distal pouch, and with the circular depression, however, there is an immediate alteration of proximal activity into the smooth, regular type II contractions mentioned above.

Repeated doses of adrenalin in both anesthetized and unanesthetized animals had essentially the same effect as the first injection. On un-

anesthetized dogs as many as 11 doses have been given at intervals of about 20 minutes without variation in the results (fig. 6). If the second dose was given during the period of augmentation of the proximal balloon and the longitudinal from a preceding injection, the effect was the same as if the activity were spontaneous, i.e., there was depression of the longitudinal, with augmentation of the circular, followed, after an interval, by augmentation of the proximal and the longitudinal over the activity preceding the injection (fig. 1, fig. 4).

On one dog, unanesthetized, a series of varying dosages was given, in an attempt to establish roughly threshold concentrations for these effects (fig. 5). It was found that 5 cc. of a 1:50,000 solution produced typical immediate augmentation of the circular, in both distal and proximal segments, followed immediately in the proximal balloons by a period in which there were high, regular type II contractions, frequently peristaltic, but that the augmentory effect on the longitudinal was lacking. Ten cubic centimeters of the same solution produced augmentory effects on both circular and longitudinal as described above. One cubic centimeter of the solution had no definite effect, although there was a slight, atypical augmentation of the circular. The latter was no greater than a similar augmentation of the circular sometimes seen when equal volumes of physiological salt were given.

In unanesthetized dogs, in addition to the typical effects described, with large doses (1 cc. of a 1:1000 solution) sometimes defecation movements were elicited, and sometimes vomiting. In dogs with intact colon (6-balloon technique) actual defecation was sometimes obtained with such doses. The description given above applies only to the effect as seen uncomplicated by these factors. In the cases described as exhibiting the typical uncomplicated effect no skeletal muscle movements could be detected, and no gross symptoms following the injection except for alterations in respiratory rate and heart rate.

Pilocarpine. A total of 35 injections was given in 14 dogs under ether or barbitol anesthesia, and 18 in 6 dogs without anesthesia. Doses varied from 1 to 10 mgm. in 0.1 per cent isotonic solution.

In all cases the effect was augmentation of both the longitudinal and circular of the distal segment, and augmentation of the proximal balloons. There was no division of the effect into phases as in the case of adrenalin, the augmentation appearing nearly simultaneously on all the records. Serial balloons in both the proximal and the distal segment showed definite peristalsis in most instances (figs. 2 and 4). Even the first contraction was frequently traceable as a wave of contraction moving toward the anus. Where peristalsis could not be detected, the contractions were high, regularly rhythmic type II contractions, resembling in character the contractions appearing during the period of proximal-longitudinal augmentation

following adrenalin. The effect was usually complete within 15 to 30 minutes.

A second injection given before termination of the effects of the first was followed by still further augmentation without appreciable latent period, and without preliminary depression in any of the records.

Pilocarpine given before the augmentory effect of adrenalin on the proximal and the longitudinal was complete was followed immediately by still further augmentation without preliminary depression or appreciable latent period (fig. 4).

Adrenalin given before the augmentory effect of pilocarpine was complete had the same effect as if given during a period of spontaneous activity, preliminary augmentation of the circular with depression of the longitudinal, followed by augmentation of the longitudinal and the proximal.

Ephedrine. A total of 8 injections was given in 4 dogs under ether or barbital anesthesia. Ephedrine sulfate, 65 mgm. (Eli Lilly & Co.) made up in about 5 cc. of isotonic salt solution was given at each injection.

The first injection was invariably accompanied by depression of the longitudinal and augmentation of the circular lasting approximately as long as the elevation of blood pressure (figs. 2 and 3). The augmented circular activity was most marked in the lower colon, and differed from the circular augmentation after adrenalin in developing more slowly and being maintained much longer. It was not possible to separate this augmentation into two parts, as in the case of adrenalin. The augmentation was characterized by well-formed type I contractions, as in the adrenalin effect. The longest duration of this effect obtained was more than 90 minutes.

A second ephedrine injection given even as late as 50 minutes following the first, failed to produce either a rise in blood pressure, or depression of the longitudinal. In three cases, where second or third injections produced a slight fall in blood pressure, the effect on the longitudinal and the proximal was augmentation rather than depression, and the distal circular augmentation was absent (fig. 3).

When adrenalin was given before recovery of longitudinal-proximal activity after a first injection of ephedrine, the augmentory effect on the longitudinal-proximal was not obtained, but the immediate circular augmentation was still elicited (fig. 2). If, however, adrenalin was administered after recovery of motility from the ephedrine effect, the typical diphasic augmentory effect on both circular and longitudinal-proximal was obtained (fig. 3). A dose of adrenalin given shortly following a second or third, non-depressant ephedrine injection elicited a response complete in all phases (fig. 3). Blood pressure response to adrenalin was not altered by previous ephedrine in either case.

Atropine. Injections of widely varying amounts of atropine sulfate up

to 250 mgm. were made in 10 anesthetized dogs. The solution used was a 0.1 per cent isotonic solution. Atropine was also given in amounts up to 5 mgm. in unanesthetized dogs.

The criteria used for complete atropinization of the peripheral mechanism were failure of response to pilocarpine, and failure of activation with very light anesthesia. This point was reached before death of the animal in only 4 cases. It was noted that when atropine paralysis of the colon was obtained, it was with moderate doses, 2 to 10 mgm. If doses of this size did not produce paralysis, larger doses also failed. In about half the cases, the 2 or 4 mgm. dose given repeatedly to reach the desired cumulative effect was followed by temporary augmentation on all the records, frequently only a single contraction.

When complete atropine paralysis was obtained, according to the above criteria, adrenalin failed to produce any activation of the longitudinal, while the immediate augmentory effect on the distal circular was preserved without apparent diminution (fig. 4). Pilocarpine at this stage failed completely to activate either the longitudinal or circular of the distal colon, or the proximal balloon.

Complete atropine paralysis was not obtained with the doses given in any of the unanesthetized animals. After small doses (2-3 mgm.) there was usually some diminution in both longitudinal-proximal and circular activity, and some diminution in the longitudinal-proximal augmentation following adrenalin, but never the complete suppression of the latter obtained in the acute animals with more complete paralysis.

Ergotamine. Ergotamine tartrate (Gynergen "Sandoz" in 1 cc. ampules containing 0.5 mgm. each) in doses of 1 mgm. was given to 6 dogs under anesthesia. The dose was repeated until the effects given below were obtained, usually after a total of 2 to 4 mgm. had been given at intervals of 30 minutes or less.

The immediate effect of such a dose was not marked. Usually there was a slight diminution in longitudinal-proximal activity, with a possible slight augmentation of the distal circular. In all cases, however, after 2 to 4 mgm. of ergotamine had been given, subsequent adrenalin injections failed completely to elicit the immediate augmentory effect on the distal circular, while the longitudinal depression followed by longitudinal augmentation was preserved (fig. 1). In place of the original immediate augmentation of circular tone and activity during the longitudinal depression, after ergotamine adrenalin produced an immediate and marked depression of tone in the circular record, concurrent with longitudinal depression. The second phase of the adrenalin effect, consisting of augmentation of the longitudinal and proximal, followed without marked alteration in time relationships or intensity. However, for a period as long as 20 minutes following increased doses of ergotamine, adrenalin

failed to augment the longitudinal also. Within 30 to 40 minutes, after ergotamine in such doses, adrenalin had recovered its augmentory effect on the longitudinal and the proximal, while the immediate augmentory effect on the circular was still entirely absent.

It was not possible, with such doses of ergotamine, to detect any alteration in the blood pressure response to adrenalin.

DISCUSSION. The differences in activity of the colon under anesthesia are in line with the observations of Miller (5) except for the important particular of longitudinal activity. Whereas the unanesthetized animal shows periodic longitudinal activity, with long intervening periods of quiet, such periodicity is almost lacking in the anesthetized animal. Under moderate anesthesia, total longitudinal activity is much greater than in the unanesthetized dog. Miller reported a stimulating after-effect of ether anesthesia. This might be interpreted as indicating stimulation masked by depression. It is possible that such an effect is sufficient to stimulate the longitudinal layer to overactivity, under light or moderate anesthesia. The possibility that the stimulation arises from our surgical procedures and the exposure of the viscera, with retention of considerable irritability in the longitudinal layer under moderate anesthesia, cannot be ruled out at present, although care was taken to protect the gut from such stimuli. Whatever the origin of the continuous longitudinal activity, it is significant that associated with it is almost complete quiet in the circular layer. Whether this indicates release of the longitudinal from the inhibiting influence of the circular mechanism, or depression of the circular from overactivity of the longitudinal mechanism, or even that the conditions cited above have opposite effects on the two layers, it is not possible to say.

That adrenalin under some conditions may exert a motor influence on the gut has been sporadically reported (6), (7), (8). Recent work indicates that previous parasympathetic stimulation is necessary in order that such an effect be obtained (9), (10), (11). However, in the colon of both the cat and the dog, under our conditions, such stimulation was not necessary. It seems to be necessary only that the longitudinal layer be capable of activity. Paralysis due to deep anesthesia, atropine, ephedrine, or large doses of ergot, rendered the longitudinal layer incapable of being augmented with adrenalin. It is not necessary, however, for the longitudinal layer to be exhibiting activity at the time, as is shown by the activation with adrenalin from complete quiet in moderately deep anesthesia.

That more than one mechanism is involved in the total response to adrenalin is suggested by the polyphasic character of the response. That the augmentory effect on the circular during the first phase is not merely contraction of the internal anal sphincter as is stated by Learmonth and

Markowitz (12) is shown by the fact that even balloons in the proximal colon show the same effect. This is true also in the colostomized dogs used in the chronic study, where the possibility of a passive effect from the sphincter is ruled out by complete separation of proximal and distal segments. In character, especially in the appearance of the well-defined type I contractions on the tone change, this first effect resembles closely the rhythmic pulsations which we described in the unanesthetized dog. It is clear that the longitudinal layer does not participate in this first effect, which is an additional point of similarity to the rhythmic pulsations. It is during this phase that the longitudinal is sharply depressed, exhibiting a reciprocity with the circular quite like the reciprocity between the longitudinal and the rhythmic pulsations of the circular in the spontaneous motility of the unanesthetized dog.

The longitudinal augmentation, associated with activity in the proximal segment, which follows the first phase, is the most striking part of the effect. Blood pressure by this time is back to normal. The entire picture in the second phase closely resembles spontaneous motility, greatly augmented, and is almost duplicated by activity following pilocarpine. Pilocarpine, injected during this phase, immediately augments still further, without grossly altering the character of the activity. Adrenalin, however, injected during this phase, first depresses the longitudinal and proximal, with augmentation of the distal circular, then augments the longitudinal and proximal above that following the first injection. By administering adrenalin at intervals before the second effect has developed, we have been able to hold out the second effect for as long as 30 minutes. It is clear that the second effect of adrenalin is antagonized by the first effect, and, further, that pilocarpine is not antagonistic, but augmentory, to the second effect.

Atropine, when we were successful in obtaining complete atropinization, further separates the first and second phases of the adrenalin response by allowing the first to develop fully while completely suppressing the second. After atropinization pilocarpine was unable to stimulate any part of the colon, while adrenalin still retained its immediate augmentory effect on the distal circular. From work on the cat's uterus, Cushny (24) states that "Pilocarpine differs from adrenalin in being antagonized completely by atropine, whether it contracts or inhibits the uterus, while the effects of adrenalin or of hypogastric stimulation are not changed in any way by atropine." In the colon, it would appear from our work that the latter part of this statement is true only of the first phase of the adrenalin effect. The second phase is as completely lost after atropinization as is the response to pilocarpine.

There is a complete dissimilarity between the latent effects of adrenalin and ephedrine, although the first effects seem to differ primarily only in

time relations. Following ephedrine, in the time allowed in our tracings, there is no augmented return of the longitudinal and proximal, but a slow recovery of the control activity. This difference between the two drugs has apparently not been observed by other workers, although Kreitmair (13) mentions that both adrenalin and ephedrine produce high tone and contractions in the excised uterus, and Kinnaman and Plant (14) state that ephedrine sometimes increased the tone and contractions in Thiry-Vella loops of the ileum. The latter call attention to "the marked similarity to the effects of epinephrine, in that the activity is decreased." The depressant effect of ephedrine on the longitudinal and the proximal seems proportional to the intensity and duration of the blood pressure effect. Where long maintained high blood pressure was obtained, depression of the longitudinal and the proximal segment was concurrent. On second or third injections, where no blood pressure rise was obtained, there was no depression of motility.

In appearance and relationship to blood pressure, it is apparent from his tracings that the first phase of the adrenalin response was observed by Bunch in 1898 (6) on the small intestine. His tracings, however, fail to show our second phase. His methods were similar to ours in that he examined the gut *in situ*, and dissimilar in that he did not attempt to separate circular and longitudinal activity. Our separation of distal colon activity into longitudinal and circular components shows clearly that in this region the depressant portion of the response to adrenalin, which is the only response usually observed with other methods, is restricted to the longitudinal layer, and to the type of circular activity associated with longitudinal activity. Circular activity of the sphincter type is augmented.

The second effect is clearly not secondary to the motor response of the circular during the first effect, for after ergot the motor response of the circular is reversed without impairing the delayed effect. The augmentation of the longitudinal, and the activity shown by the balloons during the second phase, are similar in all respects to spontaneous motility, and to motility produced by pilocarpine. The summating effect of pilocarpine during this phase, and the immediate antagonistic effect of adrenalin seem to warrant the conclusion that antagonistic mechanisms are set in activity during the first and the second phase of the response to adrenalin. That the mechanism active during the second phase is the same as that activated by pilocarpine and paralyzed by atropine seems also a justifiable conclusion.

The activity set up throughout the colon as the first effect of adrenalin is so similar in its type and in its reciprocity with the longitudinal to the rhythmic pulsations occurring during spontaneous motility in the unanesthetized dog, as to suggest a similarity in mechanism.

Assuming, with some workers (11), a specificity for adrenalin which may not exist, we should be forced to the following conclusions: 1. The first effect of adrenalin on the colon is augmentation of rhythmic pulsations throughout the colon, a purely circular, sphincter type of activity, apparently under the motor control of the sympathetics. 2. The delayed effect of adrenalin is augmentation of a reciprocal type of activity, peristalsis associated with longitudinal contractions, apparently under the motor control of the parasympathetics. Why uncomplicated stimulation of the sympathetics should be followed by activation of the parasympathetics is a question raised, but not answered, by these conjectures. Weitz and Vollers, in 1926 (8), suggested that "probably in the temporary interruption of peristaltic movement, and the diminution of tone, lies a sufficient basis for the following augmented activity." That their results

TABLE 1
Response of the colon to adrenalin

CONDITION	FIRST PHASE			SECOND PHASE		
	Proximal balloons	Distal longitudinal	Distal circular	Proximal balloons	Distal longitudinal	Distal circular
Moderate anesthesia.....	+ tone type I	—	+ tone type I	+ type II	+	— or type II
Deep anesthesia.....	+ tone type I	0	+ tone type I	0	0	0
Unanesthetized.....	+ tone type I	—	+ tone type I	+ type II	+	— or type II
After adrenalin.....	+ tone type I	—	+ tone type I	+ type II	+	— or type II
After atropine.....	+ tone type I	0	+ tone type I	0	0	0
After ergot.....	— or 0	— or 0	— or 0	+ type II	+	— or type II
After ephedrine.....	+ tone type I	0	+ tone type I	0	0	0

were specific adrenalin effects might be questioned on the basis of inadequate controls.

Data are accumulating which have been interpreted as showing that adrenalin stimulates not only the thoracico-lumbar but also the cranio-sacral apparatus. Heinekamp in 1925 (15) found that following physostigmine adrenalin produced slowing of the heart in vagotomized animals, which was antagonized by atropine. He concluded that adrenalin acts on both systems, the effect obtained being determined by relative thresholds. Smirnow and Schiroky, in 1926 (16) found exaggerated vagal beats when adrenalin was given following morphine. They concluded from this evidence that adrenalin is an amphotropic hormone, acting on whichever system is the more irritable, morphine serving to increase "vagal tone."

The opposite interpretation, in which the specificity of function of the two divisions of the autonomic system is questioned, might be considered as explaining results obtained from physical stimulation of nerves (17), (18), (19), (20), (21). It would seem, however, an economy of ideas

to withhold such interpretation until the central connections of such peripheral aggregations of autonomic fibres as the vagus and hypogastric are better known.

Following the statement by Dale in 1906 (25) of the specificity of ergot alkaloids for the motor elements associated with thoracico-lumbar innervation, most workers with this drug and its reversal of subsequent adrenalin responses have assumed that the response remaining indicated sympathetic rather than parasympathetic activity (22), (23).

For a clear-cut interpretation of our results in the light of previous work, one must assume either that adrenalin stimulates both sympathetics and parasympathetics; or, that neither the sympathetic nor the parasympathetic is, strictly speaking, motor to the colon, but that each is capable of giving rise to a particular type of motor activity, the two types being mutually antagonistic. It would seem necessary to add, to the latter alternative, that not only are the two types of activity mutually antagonistic in that they are incapable of co-existing in the same segment, but that the suppression of parasympathetic activity during that of the sympathetic is followed by a complete reversal.

Neither interpretation seems complete, nor totally satisfactory.

SUMMARY

1. Adrenalin produces in both anesthetized dogs and cats, and in un-anesthetized dogs, a diphasic augmentation of motility in the colon. During the first phase, there is augmentation of stationary circular contractions, a sphincter type of activity, throughout, with depression of longitudinal and the peristaltic type of activity. During the second phase there is complete reversal of this condition, with augmentation of the longitudinal and the peristaltic type of activity, and depression of the sphincter type.

2. Threshold doses of adrenalin produce only the first phase.

3. Atropinization antagonizes the second phase, without diminution or alteration of the first.

4. Ergotamine reverses the first phase, without alteration of the second.

5. Pilocarpine augments the second phase, without preliminary depression, and without producing any change in character of activity.

6. A second dose of adrenalin given during the second phase produces reversal of activity back to that of the first phase, followed by augmented recovery of the second phase.

7. These effects were not obtained with ephedrine, except that the total response is similar in appearance to that of the first phase of the adrenalin response.

The authors wish to express their appreciation to Prof. A. J. Carlson for advice and criticism.

BIBLIOGRAPHY

- (1) TEMPLETON AND LAWSON. 1931. *This Journal*, xcvi, 667.
- (2) LAWSON AND TEMPLETON. 1931. *Ibid.*, xci, 87.
- (3) LAWSON AND TEMPLETON. 1932. *Ibid.*, c, 362.
- (4) TEMPLETON AND LAWSON. 1931. *Journ. Lab. Clin. Med.* (in press).
- (5) MILLER. 1925. *Journ. Pharm. Exper. Therap.*, xxvii, 41.
- (6) BUNCH. 1898. *Journ. Physiol.*, xxii, 357.
- (7) SMITH, 1918. *This Journal*, xlv, 232.
- (8) WEITZ AND VOLLERS. 1926. *Zeitschr. f. d. gesammt. exp. Med.*, lv, 45.
- (9) KOLM AND PICK. 1920. *Pflüger's Arch.*, clxxxiv, 79.
- (10) TAKEDA. 1930. *Fol. Pharm. Jap.*, x, 100.
- (11) BERNHEIM AND BLOCKSON. 1932. *This Journal*, c, 313.
- (12) LEARMONTH AND MARKOWITZ. 1930. *Ibid.*, lxxxix, 686.
- (13) KREITMAIR. 1926. *Arch. f. exp. Path. u. Pharm.*, cxx, 189.
- (14) KINNAMAN AND PLANT. 1931. *Journ. Pharm. Exper. Therap.*, xliii, 477.
- (15) HEINEKAMP. 1925. *Ibid.*, xxvi, 385.
- (16) SMIRNOW AND SCHIROKY. 1926. *Zeitschr. f. d. gesammt. exp. Med.*, lv, 24.
- (17) MAY. 1904. *Journ. Physiol.*, xxxi, 260.
- (18) DALE, LAIDLAW AND SYMONS. 1910. *Ibid.*, xli, 1.
- (19) CARLSON. 1930. *Journ. Amer. Med. Assoc.*, xciv, 78.
- (20) McSWINEY AND WADGE. 1928. *Journ. Physiol.*, lxxv, 350.
- (21) ROGERS AND BERCOVITZ. 1921. *This Journal*, lvi, 257.
- (22) ROTHLIN. 1929. *Journ. Pharm. Exper. Therap.*, xxxvi, 657.
- (23) MENDEZ. 1927. *Ibid.*, xxxii, 451.
- (24) CUSHNY. 1910. *Journ. Physiol.*, xli, 233.
- (25) DALE. 1906. *Ibid.*, xxiv, 163.

AN ATTEMPT TO PRODUCE SPINAL CORD DEGENERATION IN DOGS FED A HIGH CEREAL DIET DEFICIENT IN VITAMIN A. THE INCIDENTAL DEVELOPMENT OF A SYNDROME OF ANEMIA, SKIN LESIONS, ANOREXIA AND CHANGES IN THE CONCENTRATION OF BLOOD LIPOIDS¹

M. M. SUZMAN,² GULLI LINDH MULLER AND C. C. UNGLEY³

From the Massachusetts General Hospital, the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston

Received for publication April 18, 1932

The beneficial effect of large amounts of whole liver upon the neurological phenomena of subacute combined degeneration of the cord was demonstrated by Minot and Murphy (1927) and has subsequently been confirmed by others. Ungley and Suzman (1929) have suggested that there may be a factor in liver, distinct from the principle effective in pernicious anemia, which influences the nervous system beneficially and that only a part of the neurological improvement is dependent upon alleviation of anemia and gain in strength. In order to determine whether such a separate factor exists, an attempt has been made to produce in dogs a condition simulating subacute combined degeneration in man.

It was thought that this problem could be approached from a nutritional standpoint, because neurological lesions similar to those of pernicious anemia occur in pellagra, lupinosis, ergotism and the "central neuritis of Jamaica," conditions usually regarded as being dependent on dietetic anomalies. Furthermore, Castle and co-workers (1930) have demonstrated that Addisonian pernicious anemia can be regarded as a dietary deficiency disease conditioned by the state of the gastro-intestinal tract.

Although the nervous lesions which develop with a deficiency of vitamin B are usually confined to the peripheral nerves, lesions resembling those found in subacute combined degeneration may occur in the spinal cord (Vedder and Clark, 1913; Gildea et al., 1930). Furthermore, Eijkman

¹ The expenses of this investigation have been defrayed by the Rockefeller Foundation. We offer thanks to Charles E. Walker, A. Bloomberg, and B. Miller for their assistance in the care of the animals. We are especially grateful to Dr. George R. Minot and Dr. James H. Means for their kindly criticism.

² Medical Research Fellow, Rockefeller Foundation, and Research Fellow in Medicine, Massachusetts General Hospital and Harvard Medical School, Boston.

³ Medical Research Fellow, Rockefeller Foundation, and Research Fellow in Medicine, Massachusetts General Hospital and Harvard Medical School, Boston.

(1897), Funk (1914), McCarrison (1928) and Vogt-Moller (1931) have shown that the central nervous system symptoms are attributable, not to organic change, but rather to an intoxication of the system from an interference with metabolism or from the ingestion of a toxic factor. Diets deficient in vitamin B were therefore considered unsuitable for the purposes of this experiment.

However, since neurological manifestations indicative of spinal cord involvement, supported in some by histological findings, have been produced in animals on vitamin A deficient diets (Steenbock et al., 1921) usually containing a high proportion of cereal (Hart et al., 1916; Mellanby, 1926, and Hughes et al., 1929); an attempt was made to produce spinal cord degeneration in dogs by means of a similar diet. The inclusion of cereal has been regarded as supplying a positive toxic agent or "toxamin" (Mellanby 1930) inimical to the central nervous system. Although the attempt proved unsuccessful, there developed certain incidental and unexpected manifestations, a description and discussion of which form the main subject of this paper.

EXPERIMENTAL METHODS. Eight adult dogs were fed a basal diet consisting of rolled oats 76.8 per cent, sugar 12.8 per cent, lard 6.4 per cent, bone ash 2.4 per cent, salt mixture⁴ 1.6 per cent (Cowgill, 1921) and 10 drops three times weekly of Viosterol in Oil, 250 D, N.N.R. This diet contains protein 12.3 per cent, carbohydrate 64.6 per cent, fat 11.9 per cent and mineral content 5.5 per cent. It was considered that this diet contained sufficient of all known vitamins excepting vitamins A and C. Reasons for the omission of the latter will be given later, and the possible shortcomings of the vitamin content in regard to the vitamin B complex will be discussed forthwith. Two of the animals received in addition a source of vitamin A, in the form of mammalian liver oil or of cod liver oil concentrate. Inasmuch as the object was to produce a toxic effect by excess of cereal, in addition to the effect of avitaminosis A, the dogs were given as much of the above mixture as they would eat. In an attempt to increase the toxic effect still further, an extract of oatmeal was injected into one of the dogs.⁵

⁴ The salt mixture consists of sodium chloride 10 grams, calcium lactate 4 grams, magnesium citrate 4 grams, ferric citrate 1 gram, iodine-potassium iodide solution 10 minims.

⁵ This was done in view of the work of Teruchi and co-workers (1929). These investigators, having first demonstrated a quantitative relationship between the amounts of polished rice necessary to produce polyneuritis and those of anti-neuritic vitamin sufficient to prevent it, and having subsequently prepared from polished rice an alcoholic extract, which on injection into animals deprived of vitamin B, would produce polyneuritis, concluded that polished rice contains a neuro-toxic factor, the action of which is neutralized by the administration of vitamin B. Thus, applying this rationale to our experiments, large amounts of finely ground whole oatmeal were extracted several times with absolute alcohol, which was then evaporated down in vacuo. The residue, consisting of a dark brown oil, was used for injection into one of the animals.

The animals were kept in individual cages, fed once daily, exercised periodically and weighed at intervals.

In view of the fact that subacute combined degeneration of the cord is almost invariably associated with pernicious anemia, the red blood cell count and hemoglobin concentration were observed with regularity. Further, in view of the paucity of blood lipid studies during vitamin A deficiency, opportunity was taken to note any possible fluctuations in the blood of the concentration of cholesterol and lecithin. Determinations of the red and white blood cell, hemoglobin, cholesterol and lecithin were made once a week on oxalated venous blood obtained from the femoral vein under uniform conditions, at the same time in the morning in the post-absorptive stage. The cholesterol was determined by Bloor's saponification method (1922), and the lecithin phosphorus according to the method of Whitehorn (1924), both methods found to give consistent values in duplicate when carried out under standard conditions.

Since it is known that complete vitamin deprivation of the body tissues is brought about less readily and with less regularity under given conditions in adult than in young animals, owing to variations in stores and requirements, it was thought desirable to ascertain whether or not vitamin A exhaustion had occurred, by estimating the concentration of this vitamin in the organs after death. For this purpose portions of liver, brain, kidney and spleen removed at autopsy were placed in 50 cc. of a 40 per cent aqueous solution of potassium hydroxide. After saponification, the material was extracted three times with petroleum ether. After washing with water several times until there was no tendency to form an emulsion, and after dehydrating by means of anhydrous sodium sulphate, the petroleum ether layer was evaporated to dryness under partial pressure in an atmosphere of carbon-dioxide. This residue, consisting of the unsaponifiable fraction, was dissolved in a known amount of chloroform and assayed for its vitamin A content by the antimony trichloride method of Carr and Price (1926), using the Rosenheim-Schuster modification of the Lovibund tintometer, the results being expressed in blue units per gram of tissue "per cm. cube," according to the method of Moore (1929).

ANALYSIS OF FINDINGS. Of the eight dogs used, three died before the completion of the experiment, in one case due to distemper, in another due to the effects of the injections of oatmeal-extract, and in the third following the removal of a small portion of the liver for assay of its vitamin A content. In the five remaining dogs, there were no significant differences in the symptomatic manifestations or blood findings between the three dogs fed only the basal diet, and those which received vitamin A in addition. The findings in these dogs, therefore, may be grouped together.

These five dogs lived for periods of from twenty-three to thirty-five weeks on the experimental diet. They ate well for periods varying from eleven

to nineteen weeks and continued to gain weight for periods of from five to eleven weeks. In three, the weight commenced to decline coincident with the onset of anorexia, whereas in two the loss of weight preceded the loss of appetite by some weeks, attributed to attacks of diarrhea. It may be mentioned here that one dog, although dying prematurely as the result of an operation nineteen weeks after the beginning of the experiment, had already shown anorexia and loss of weight commencing as early as the fifth week.

All the five dogs developed dermatitis with loss of hair and in four of these skin ulcers were also present. These skin lesions appeared earlier and were more severe in the two dogs receiving vitamin A. Three types of lesions were present. First, a dry scaly condition with loss of hair; secondly, the occurrence of slightly raised reddened areas on the bony prominences of the limbs and elsewhere; and thirdly, the subsequent development in these latter situations of round or oval ulcers. These were somewhat punched-out and had a flat, fairly clean base from which there was a small amount of brownish discharge. At first about one-half inch in diameter, they had increased at the time of death to from one to two inches across and were deep enough to expose the underlying hard structures. In the later stages the edges of the ulcers became somewhat raised and rounded. It is noteworthy that these lesions showed little gross evidence of inflammatory reaction, there being no signs of local congestion, tenderness, edema or purulent secretion. Repeated treatment with antiseptic lotions and with a proprietary mange cure, although causing some temporary improvement in the scaly skin eruption and allaying irritation, failed to affect the progress of the ulcers.

A purulent discharge was noted in the eyes of the two dogs receiving vitamin A and in one being fed the basal diet alone, but in none was there evidence of xerophthalmia. Apart from some unsteadiness in the latter stages, probably due to extreme weakness, there were no neurological manifestations.

The two female dogs, neither of which received vitamin A, developed a condition of continuous heat, as shown by their attitude towards male dogs, and which persisted to within two weeks of death.

Anemia developed in all the five dogs in which the experiment was completed, the hemoglobin concentration falling to a slightly greater extent than the red blood cell count. This was noticeable by the twelfth, eleventh and twentieth week respectively in the three dogs not receiving vitamin A, whereas in those receiving this vitamin, the onset of the anemia occurred during the twentieth week. In one dog in which the experiment was not completed, some degree of anemia was noticeable by the fifteenth week.

A terminal rise of the cholesterol content of the blood occurred in three dogs, and a fall in one, which change in each case was coincident with the

development of the anemia. In the remaining dog the cholesterol values showed no significant alteration. The concentration of lecithin underwent similar fluctuations in all except one dog in which no significant change was noted. The course of two of these animals is illustrated in the charts.

A careful microscopical study of the nervous system failed to reveal any significant pathological changes. The myelin sheaths were stained by means of the Spielmeyer, Ioyez, Pal-Weigert and Scharlach-R methods,

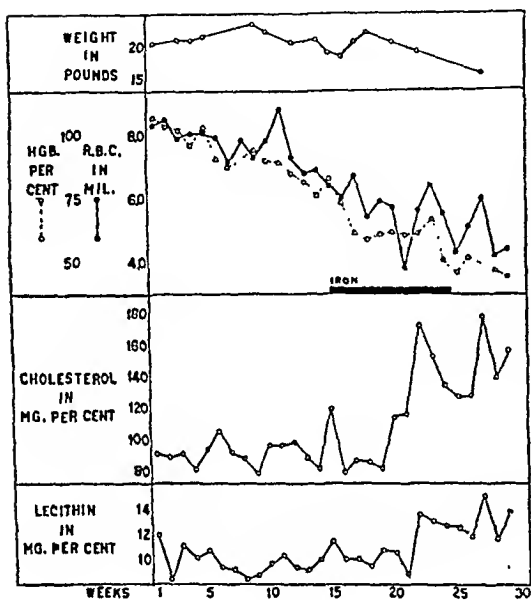


Chart 1

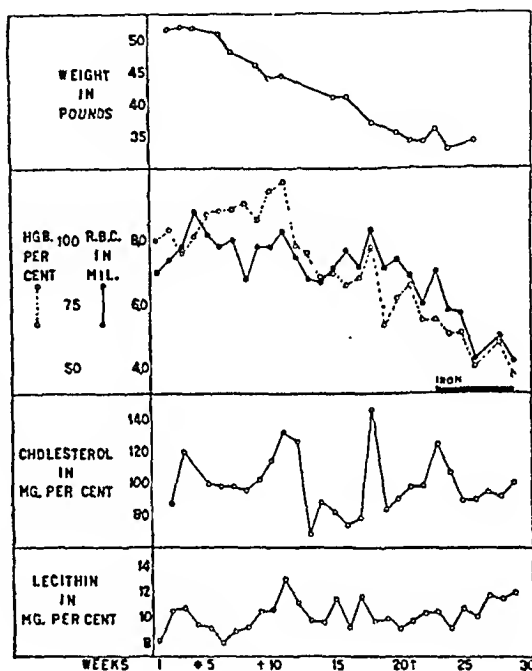


Chart 2

Chart 1. Body weight, hemoglobin concentration, red blood cell count and lipid content of blood in a dog on basal diet without vitamin A. Skin rash and ulcers appeared during the last two weeks of life.

Chart 2. Body weight, hemoglobin concentration, red blood cell count and lipid content of blood in a dog receiving the basal diet with vitamin A.

* First appearance of skin rash.

+ First appearance of purulent conjunctivitis.

↑ First appearance of skin ulcers.

the axis cylinders by the technique of Davenport (1929), the nerve cells by means of cresyl violet, and the hemotoxylin-and-eosin method was used as a general stain. The only positive histo-pathological finding in the nervous system was the presence, in the cerebral cortex, of degenerative swelling of the oligodendroglia, which condition is considered by Wolff, Reed and Cobb (1928) to be indicative of a severe intoxication.

Apart from the presence of terminal broncho-pneumonia and of hyper-

plasia of the bone-marrow, no significant gross or microscopical changes were evident. The body fat, when present, was snow-white in colour.

The tissues of the animals receiving the basal diet alone, and in which the experiment was completed, contained no vitamin A, whereas those receiving this vitamin showed an abundance in the liver and small amounts in the kidney and spleen.

DISCUSSION. Two chief points of interest arise. First, the fact that no neurological changes occurred, and secondly, the occurrence of anorexia, emaciation, skin lesions, anemia and alterations in the concentration of the blood lipoids. Since these manifestations occurred whether or not vitamin A was given, it is obvious that some other deficiency must have been present in the basal diet. Therefore, before the significance of these results can be discussed, it is necessary critically to consider the composition of the diet.

It was estimated that the *caloric value* of the diet was approximately 120 calories per ounce of mixture, and that during the periods of good appetite the daily consumption was half an ounce per pound body weight. The daily caloric intake (approximately 60 calories per pound body weight), therefore, was far in excess of the basal requirements (approximately 35 calories per pound body weight). However, during the latter part of the experiments, when the appetite had diminished considerably, the caloric intake was less than the basal requirements.

The *protein* content of the diet was 12.4 per cent which, for biologically adequate protein, is generally accepted as being sufficient for the maintenance of health in adult animals. That the oat kernel can furnish all the essential nitrogenous units provided its concentration of protein is adequate, namely, 10 per cent, has been pointed out by Osborne and Mendel (1920). In the nutrition of the adult human, the adequacy of the oat protein has been demonstrated by Sherman and co-workers (1919). Moreover, Luers and Siegert (1924) have shown that the protein of oatmeal contains very liberal amounts of the essential amino-acids, and Smith and Hendrick (1926) have demonstrated that, when properly supplemented with accessory food factors, it is just as satisfactory as casein in the nutrition of the rat.

The *salt mixture* has already been shown by Cowgill (1921) and by other investigators (Gildea et al., 1930) to be adequate for the maintenance of health in dogs. The iron content of the basal diet, excluding the small amounts present in oatmeal (3.8 mgm. per 100 gm.), was estimated at 0.0184 per cent. That is to say, the amount of metallic iron ingested during the periods of normal appetite was equivalent to 2.7 mgm. per pound body weight daily, or an average of 98 mgm. per dog daily. In view of the development of anemia, and to obviate the possibility of an iron deficiency dependent on the diminished food intake in the latter parts of the experiments, additional amounts of iron were administered in the form of iron

and ammonium citrate (10 gr. daily, equivalent to 108 mgm. of metallic iron). That these amounts were sufficient is borne out by the work of Whipple and Robscheit-Robbins (1930), who have shown that for maintenance of the hemoglobin level dogs require 20 mgm. of metallic iron daily, and further, that the optimum necessary for regeneration of hemoglobin in anemia due to blood loss is an additional 40 mgm. of metallic iron daily. Moreover, according to Riecker (1930), the optimum daily amount of metallic iron required by the dog for regeneration of hemoglobin after bleeding is 57.5 mgm.

With regard to the presence in oatmeal of the *vitamin B complex*, it is well known that abundant anti-neuritic vitamin B₁ is present. Although Smith and Hendrick (1926) have shown that oatmeal is relatively deficient in the growth-promoting heat-stable factor of vitamin B complex, we originally believed that the large proportion of oatmeal in the diet would supply sufficient of this factor for maintenance of health in adult animals, and that it would therefore be unnecessary to supplement the basal diet with some other source of this factor, such as yeast. Moreover, there is evidence that less of the heat-stable component is required when the protein content of the diet is relatively low (Hartwell, 1924), as was the case in our basal diet. In the light of subsequent events in our experiments, however, it seems probable that there was present in the basal diet given our dogs a relative deficiency of some portion of the vitamin B complex other than the anti-neuritic. Since none of the dogs developed any lesions in the mouth, it is unlikely that the diet was deficient in the so-called "anti-black-tongue" factor, described by Goldberger and associates (1928). Nevertheless, there may have been lacking still other components of the vitamin B complex, the composite nature of which has been demonstrated by several investigators (Hunt 1928, 1931; Reader, 1929, 1930; Williams and Waterman, 1928; Chick and Copping, 1930; Coward et al., 1929).

Most sources of vitamin C, such as orange and lemon juice, contain significant amounts of vitamin A (Willimott, 1928). For this reason, and because there is experimental evidence to show that dogs on a diet lacking in vitamin C do not develop scurvy for periods of from seven to ten months (Lavialle, 1927), it was decided to omit this factor from the basal diet.

As already stated, *vitamin D* was administered in the form of irradiated ergosterol (Viosterol). Oatmeal, as in the case of other cereals, contains abundant *vitamin E*.

Absence of neurological changes. Since several observers, using a vitamin A deficient diet usually containing a high proportion of cereal, have noted neurological symptoms in animals and in some have demonstrated pathological changes in the central nervous system, the question arises as to why no such changes occurred in our dogs. There are several possible explanations.

In the majority of previous experiments young animals were used. We desired to make a comparison with human subacute combined degeneration of the cord, which occurs only in adults, so that grown dogs were purposely chosen. The explanation might lie in the fact that adult animals are less susceptible to vitamin A deficiency than are young growing animals, the stores of which are more rapidly depleted, and, therefore, spinal cord degeneration may require a much longer time to develop than is the case in young animals.

It is not improbable that spinal cord degeneration does not develop until complete exhaustion of vitamin A has been present for a considerable period of time. Since death occurred from causes not attributable entirely to vitamin A deficiency, it is almost certain that the animals would have lived longer had this vitamin been the only limiting factor in the basal diet. That the vitamin A in these dogs had become exhausted only a short time before death is suggested by the observation that in one of the animals which died after nineteen weeks, the liver still contained appreciable amounts of this vitamin.

It is possible, moreover, that in the presence of other deficiencies the specific effects of avitaminosis-A may fail to become manifest. In addition, it is likely, in view of the suggestion of Underhill and Mendel (1928) and of Wolbach and Howe in connection with vitamin C (1926), that the breaking down of tissues which accompanied the emaciation may have liberated some vitamin A. Further, the small amounts of oatmeal ingested in the latter half of each experiment as a result of the anorexia, may have been insufficient for the development of cord degeneration, since, according to the observations of Mellanby (1926, 1930), the production of such lesions seems to depend not only upon the lack of vitamin A, but also upon the toxic effect of large amounts of cereal in the diet.

Consideration of positive findings. The significant changes which occurred during the administration of the diet, were: anorexia, loss of weight, skin lesions, eye infections, prolonged heat, anemia and changes in the lipoid content of the blood.

Since these manifestations occurred irrespective of whether or not the dogs were receiving vitamin A, it is clear that some other deficiency or adverse factor must have been present in the basal diet.

Although *anorexia* developed gradually after periods varying from ten to nineteen weeks, the dogs ate heartily before this time and gained weight for varying periods, the diet apparently being palatable and of sufficient caloric value. It has been shown that the striking anorexia, which follows deprivation of the vitamin B complex in dogs and other animals, is a characteristic of lack of the antineuritic component (Bing and Mendel, 1929; Cowgill et al., 1931), but the diet in the present experiments contained an abundance of this latter constituent. The loss of appetite which develops

in the dogs deprived of the heat-stable, "black-tongue-preventing" factor of the vitamin B complex has been shown by Goldberger and associates (1928) to be attributable to the occurrence of severe mouth lesions, which, as has already been stated, were totally absent in our dogs. Nevertheless, the anorexia in our dogs might have been due to the relative deficiency in oatmeal of the heat-stable growth-promoting factor of Smith and Hendrick (1928), the lack of which has been shown to cause loss of appetite in rats.

The *emaciation* was obviously due, in part at least, to the small food intake dependent on the anorexia, which latter, as suggested above, was perhaps the result of some deficiency in the diet. However, it has been shown that not all the loss of weight which occurs during vitamin deprivation is due to anorexia and low food intake. For example, in deprivation of the vitamin B complex, Stucky and Rose (1929) have shown that loss of weight of dogs deprived of this vitamin was greater than that of control animals ingesting exactly the same amounts of food and water.

With regard to the occurrence of *skin lesions* in animals on deficient diets, it may be noted that in the experiments of Goldberger and associates (1928), dogs, which developed so-called "black-tongue" on a diet deficient in the heat-stable component of the vitamin B complex, showed no dermatitis except in the scrotum. However, on a similar diet, Gildea, Kattwinkel and Castle (1930) reported the occurrence of a generalized mange-like scaly skin eruption with loss of hair. Underhill and Mendel (1928) while experimenting with the Chittenden-Underhill diet noted skin rash and loss of hair in dogs, the occurrence of which seemed independent of the development of the typical mouth lesions, and which could be prevented and sometimes alleviated by the administration of yeast. It is well known that rats fed a diet deficient in the heat-stable components of the vitamin B complex, not only fail to grow, but usually develop a typical dermatitis with loss of hair (Lavialle, 1927; Chick and Roscoe, 1927). The work of Cowgill, Stucky and Rose (1929) is of especial interest in that, during the course of the administration of a diet deficient in the vitamin B complex, dogs developed characteristic symmetrical ulcers on bony prominences which, from their description, are very similar to those noted in our dogs. These various observations suggest that the skin lesions in our dogs may have been due, in part at least, to a deficiency of some component of the vitamin B complex other than vitamin B₁.

The *eye infections*, consisting of a simple purulent conjunctivitis, obviously cannot be attributed to vitamin A deficiency, since two of the animals were receiving this vitamin in their diet and at death had an abundance in the liver. Although the cause of these eye lesions is not clear, it is probable that they were the result of direct extension from the several septic skin lesions which were particularly marked in these dogs. In view of the possible deficiency of some portion of the vitamin B complex in the basal diet,

it is of interest that Goldberger and Wheeler (1928) noted ophthalmia in a proportion of dogs fed a diet deficient in the heat-stable factor of the vitamin B complex. It should be recalled that in our experiments xerophthalmia did not occur in the animals deprived of vitamin A. However, as has been shown in rats by Osborne and Mendel (1921), in guinea pigs by Wolbach and Howe (1928) and in monkeys by Tilden and Miller (1930), the development of this condition during vitamin A deprivation is not a constant feature. This is especially the case in mature animals, either because they have greater stores of the vitamin, or because their requirements are less (Sherman and Smith, 1931).

Concerning the occurrence of *continuous heat* in the two female dogs deprived of vitamin A, it is difficult to understand why this should have happened. Heat in the dog, a monoestrous animal, occurs for a short period, from seven to ten days (Marshall and Jolly, 1905; Evans and Cole, 1927), in each breeding season, of which there are two annually, one each in the spring and autumn. In these two animals, however, active heat, as judged by their attitude toward male dogs was present from August to February, a period of six months. There is no evidence that vitamin A deficiency causes prolonged oestrus in other animals as rats (Evans, 1928) the cornified vaginal cells usually present being most probably an expression of the general cornification of epithelium which occurs in the course of this deficiency (Wolbach and Howe, 1925). We are unable, therefore, to explain the occurrence of protracted heat in our dogs on the basis of a lack of vitamin A. It also cannot be said whether or not the other shortcomings of the basal diet were involved in the production of this unusual phenomenon.

The development of a severe *anemia* in all the dogs in which the experiments were completed is considered the most important positive finding. As has already been stated, the diet contained sufficient minerals to maintain health in dogs, and moreover, the addition of an extra amount of iron did not prevent the progress of anemia, so that a deficiency in the diet of this inorganic constituent cannot be held responsible. However, in view of the work of Hart and co-workers (1925) who have shown that the utilization of iron is dependent on the presence in the diet of certain organic substances, such as cabbage, alcoholic extract of cabbage or chlorophyll, it is possible that the lack of some such substance from our diet may have played a rôle in the development of anemia by rendering the ingested iron unavailable for hematopoiesis. If this substance is a pigment, as has been inferred by those investigators, then the possibility of it having been absent from our diets is suggested by the fact that the small amounts of fat present in our dogs at autopsy were devoid of pigment, being snow-white in colour. Furthermore, Mouriquand and associates (1925) have made the important observation that the iron content of the blood in guinea pigs

with scurvy is considerably lower (0.21 to 0.4 gm. per kgm. body weight) than that of normal animals (0.53 gm. per kgm. body weight), and that the low concentration of iron in the blood returns to normal shortly after the resumption of an anti-scorbutic diet. It is therefore possible that, although adequate amounts of iron were provided, the lack of some factor in our diets may have been instrumental in preventing the absorption or utilization of this metal.

Since, as far as we are aware, there are no records in the literature of blood studies on dogs fed oatmeal for prolonged periods of time as the sole source of protein, it is not possible to say whether or not the anemia could be attributed to a poor quality or quantity of the protein. This, however, is very unlikely, since the oat protein is biologically adequate for the rat and the adult human.

The relation of the vitamins to blood formation has been investigated from both the experimental and the clinical points of view. Experimentally it has been shown by several observers (Happ, 1922; Cramer et al., 1922; Turner, 1930, and others) that anemia can not be produced in rats on a diet lacking vitamin A. Although Koessler, Maurer and Loughlin (1926) believed that the anemia they produced in rats was due to lack of vitamin A, Simmonds, Becker and McCollum (1927) pointed out that the diet of the former workers was deficient not only in vitamin A but also in vitamin E and iron. Sure, Kik and Walker (1929, 1931) found no noteworthy disturbances of hemopoietic function in rats during avitaminosis-A, but that infection and inanition may complicate the picture during the stage of ophthalmia. No constant changes have been found in the bone marrow of rats fed a vitamin A deficient diet, although in some animals which have survived for a long period of time, there occurs an almost complete replacement of the bone marrow by fibrous tissue (Findley and Mackenzie, 1922). The influence of vitamin A deficiency on the hemopoietic system in man is problematic. However, general nutritional defects, associated with a lack of vitamin A severe enough to produce keratomalacia may be associated with anemia, although numerous instances of the eye condition have been reported without anemia. Keefer and Yang (1929) conclude that, in man, diets deficient in vitamin A may produce keratomalacia without existence of anemia, although the latter may develop from a nutritional defect where vitamin A is only one of the deficient factors.

A number of observers have found that no anemia occurs in rats or in dogs fed diets deficient in the whole vitamin B complex. Sure, Kik and Walker (1929) and Rose, Stucky and Mendel (1930) using rats and Stucky and Rose (1929) using dogs, found that there was actually an increase of hemoglobin which was attributed to anhydremia. However, it should be pointed out that, owing to the early development of polyneuritis, the dogs of Stucky and Rose received the deficient diet for periods considerably

shorter (from 7 to 10 weeks) than those which elapsed before the onset of anemia in our animals (from 11 to 16 weeks) which were receiving adequate vitamin B₁. In man the consensus of opinion seems to support the view that lack of vitamin B₁ does not produce anemia. The absence of anemia in beri-beri was commented upon by Vedder (1913), and Keefer and Yang (1929) in a series of cases of this disease did not find anemia in the majority of patients, although it might be quite marked when, in addition, other nutritional defects are present.

With regard to the effect of deficiency of vitamin B₂, Sure, Kik and Smith (1921) found that in rats a considerable proportion of animals developed a severe anemia, which was especially prevalent in those which had dermatitis. In pellagra (Hillman, 1913) anemia of a variable degree exists, but it is not a prominent feature of the disease, which may be present for a considerable time without leading to anemia. In this connection it is of interest to note that the so-called "pernicious anemia of pregnancy" and "tropical anemia" in India may be cured according to Wills (1931) by either "marmite" or by commercial liver extract,⁶ both potent sources of vitamin B₂, while vitamins A and C are ineffective.

Scurvy produced experimentally in guinea pigs is often accompanied by anemia (Meyer and McCormick, 1928). It is well known also that in human scurvy, anemia may occur, which is benefited specifically by the administration of food rich in vitamin C (Mettier et al., 1930). For reasons which have already been mentioned, vitamin C was omitted from the basal diet, and although there were no manifestations or pathological evidence of scurvy in these animals, the possibility is nevertheless to be considered that the anemia may have been due to the lack of this factor.

On account of the marked anorexia which developed in the later stages of the experiments, it may be argued that the anemia could have been attributed so the inanition resulting from a deficient food intake. However it has been pointed out by Benedict (1915) that in man and dogs, starvation does not give rise to anemia. Furthermore, this writer claimed that anemia was not a feature during partial starvation over a long period of time in spite of the development of extreme emaciation. It is not unlikely, therefore, that the anemia in our dogs may have been due to a lack of vitamin B₂ or some other component of the vitamin B complex other than the anti-neuritic.

The knowledge of the nutritional factors determining the level of the lipoids in the blood is scanty, most of the work having been directed toward an attempt to produce a hypercholesteremia in man and animals by food rich in fat and lipoids. Low cholesterol diets have shown variable results

⁶ "Hepatopson" and Liver Extract "B.D.H.", both presumably potent for pernicious anemia.

occasionally causing a decrease of the cholesterol level in the blood while in a number of cases no change could be demonstrated.

In view of the low food intake in the later stages of our experiments, it is noteworthy that in starvation or inanition it has been shown that there may be an increase of the fat and lipoids in the blood, followed by a hypocholesteremia when the fat depots are exhausted. This subject has recently been reviewed by Peters and Van Slyke (1931) and Muller (1930). Further, in undernourishment during the World War, Rosenthal and Patrzek (1929) found a hypocholesteremia, while Strathman-Herweg (1920) obtained normal values in undernourished children.

Since there may possibly have been a somewhat low protein content in our basal diet, the work of Barker (1930) concerning the relation between the cholesterol level of the plasma and a disturbed nitrogen balance may be significant. Dogs, fed a diet deficient in nitrogen but otherwise adequate, showed an initial gradual increase followed by a terminal decline in the cholesterol content of the blood.

It has been suggested that the metabolism of cholesterol is influenced by the vitamin A content of the diet (Kimura, 1928; Liang and Wacker, 1925). However, in our experiments, since the lipid changes in the blood occurred irrespective of the vitamin A content of the diet, the results can not be ascribed to this factor. In pellagra there has been reported hypercholesteremia (Peters and Van Slyke, 1931), whereas lack of vitamin C is said to exercise no influence upon the cholesterol content of the blood in guinea pigs (Moriquand et al., 1925), while in human scurvy the cholesterol level of the blood is below normal (Sokoloff, 1924).

From the above considerations, although inanition and the absence of vitamin C from the diet can not be definitely excluded as contributory causes in the production of some of the manifestations described, it seems probable that the responsible limiting factor in the basal diet was one or more of the components of the vitamin B complex, other than the anti-neuritic. However, since the specific effects of deprivation of each of the different components have not as yet been clearly demonstrated in dogs, it is not possible to state to what extent the findings in these experiments may have been dependent on such a deficiency.

SUMMARY

1. An attempt to produce spinal cord degeneration in adult dogs by feeding a diet abundant in cereal and lacking in vitamin A proved unsuccessful. Possible reasons are given for this failure.

2. Irrespective of whether or not the dogs were receiving vitamin A, there occurred, however, a syndrome characterized by anorexia, loss of body weight, dermatitis, skin ulcers, anemia and changes in the concentration of the blood lipoids.

3. It is assumed, therefore, that, in addition to the absence of vitamins A and C, the basal diet was deficient in some other essential factor, which, on indirect evidence, is considered to be probably some portion of the vitamin B complex, other than vitamin B₁.

4. Although administration of additional iron failed to prevent the further development of the anemia, the possibility of failure of absorption or of utilization of this metal is nevertheless to be considered.

5. Continuous heat, as judged by their attitude toward male dogs, was a feature in the two female dogs deprived of vitamin A.

BIBLIOGRAPHY

- BARKER, M. H. 1931. *Proc. Soc. Exp. Biol. and Med.*, xxviii, 1081.
 BENEDICT, F. G. 1915. *Carnegie Inst. of Washington. Pub. no.* 203.
 BING, F. C. AND L. B. MENDEL. 1929. *Journ. Nutri.*, ii, 49.
 BLOOR, W. R., K. F. PELKAN AND D. M. ALLEN. 1922. *Journ. Biol. Chem.*, lii, 191.
 CARR, F. H. AND E. A. PRICE. 1926. *Biochem. Journ.*, xx, 497.
 CASTLE, W. B., W. C. TOWNSEND AND C. W. HEATH. 1930. *Amer. Journ. Med. Sci.*, lxxx, 305.
 CHICK, H. AND M. H. ROSCOE. 1927. *Biochem. Journ.*, xxi, 698.
 CHICK, H. AND A. M. COPPING. 1930. *Biochem. Journ.*, xxiv, 1764.
 COWARD, K. H., K. M. KEY AND B. G. E. MORGAN. 1929. *Biochem. Journ.*, xxiii, 695.
 COWGILL, G. R. 1921. *This Journal*, lvii, 420.
 COWGILL, G. R., C. J. STUCKY AND W. B. ROSE. 1929. *Arch. Pathol.*, vii, 197.
 COWGILL, G. R., H. A. ROSENBERG AND J. ROGOFF. 1931. *This Journal*, xevi, 372.
 CRAMER, W., A. H. DREW AND J. C. MOTTRAM. 1922. *Proc. Roy. Soc. (London)* Series B, xciii, 449.
 DAVENPORT, H. A. 1929. *Anat. Rec.*, xlv, 79.
 EIJKMAN, C. 1897. *Virchow's Arch.*, cxlix, 187.
 EVANS, H. M. AND H. H. COLE. 1927. *Anat. Rec.*, xxxv, Abs.
 EVANS, H. M. 1928. *Journ. Biol. Chem.*, lxxvii, 651.
 FINDLAY, G. M. AND R. D. MACKENZIE. 1922. *Journ. Path. and Bact.*, xxv, 402.
 FUNK, C. 1914. *Zeitschr. Physiol. Chem.*, lxxxix, 378.
 GILDEA, E. F., E. E. KATTWINKEL AND W. B. CASTLE. 1930. *New Eng. Journ. Med.*, ccii, 523.
 GOLDBERGER, J. AND G. A. WHEELER. 1928. *U. S. Pub. Health Repts.*, xliii, 172.
 HAPP, W. M. 1922. *Bull. Johns Hopkins Hosp.*, xxxiii, 163.
 HART, E. B., W. S. MILLER AND E. V. MCCOLLUM. 1916. *Journ. Biol. Chem.*, xxv, 239.
 HART, E. B., H. STEENBOCK, C. A. ELVEHJEM AND J. WADDELL. 1925. *Journ. Biol. Chem.*, lxxv, 67.
 HARTWELL, G. A. 1924. *Biochem. Journ.*, xviii, 785.
 HILLMAN, O. S. 1913. *Amer. Journ. Med. Sci.*, cxlv, 507.
 HUGHES, J. S., H. F. LIENHARDT AND C. E. AUBEL. 1929. *Journ. Nutri.*, ii, 183.
 HUNT, C. H. 1928. *Journ. Biol. Chem.*, lxxviii, 83; lxxix, 723.
 HUNT, C. H. AND W. WILDER. 1931. *Journ. Biol. Chem.*, xc, 279.
 KEEFER, C. S. AND C. S. YANG. 1929. *Nat. Med. Journ. of China*, xv, 419.
 KIMURA, H. 1928. *Acta Scholae Med. Univ. Imp. Kioto*, xi, 319.

- KOESSLER, K. K., S. MAURER AND R. LOUGHLIN. 1926. *Journ. Amer. Med. Assoc.*, lxxxvii, 476.
- LAVIALLE, P. 1927. *Bull. Soc. Chim. Biol.*, ix, 208.
- LIANG, B. AND L. WACKER. 1925. *Biochem. Zeitschr.*, clxiv, 371.
- LUERS, H. AND M. SIEGERT. 1924. *Biochem. Zeitschr.*, cxliv, 467.
- MARSHALL, F. H. A. AND W. A. JOLLY. 1905. *Phil. Trans.*, cxcviii, 99.
- MCCARRISON, R. 1928. *Ind. Med. Res. Mem.*, x, 146.
- MELLANBY, E. 1926. *Journ. Physiol. Proc.*, p. 61.
1930. *Brit. Med. Journ.*, i, 677.
- METTIER, S. R., G. R. MINOT AND W. C. TOWNSEND. 1930. *Journ. Amer. Med. Assoc.*, xcv, 1089.
- MEYER, A. W. AND L. M. MCCORMICK. 1928. *Studies on scurvy*. Stanford Univ. Press, ii, 199.
- MINOT, G. R. AND W. P. MURPHY. 1927. *Journ. Amer. Med. Assoc.*, lxxxix, 759.
- MOORE, T. 1929. *Biochem. Journ.*, xxiii, 1267.
- MORICQUAND, G., A. LEULIER AND P. MICHEL. 1925. *Compt. Rend. Acad. Sci.*, clxxx, 1925; *Compt. Rend. Soc. Biol.*, xcii, 269.
- MULLER, G. L. 1930. *Medicine*, ix, 119.
- OSBORNE, T. B. AND L. B. MENDEL. 1920. *Journ. Biol. Chem.*, xli, 275.
1921. *Journ. Amer. Med. Assoc.*, lxxvi, 905.
- PETERS, J. P. AND D. D. VAN SLYKE. 1931. *Quantitative clinical chemistry*. Vol. I, 218. Williams & Wilkins Co.
- READER, V. 1929. *Biochem. Journ.*, xxiii, 689.
1930, *Ibid.*, xxiv, 77.
- RIECKER, H. H. AND M. E. WINTERS. 1930. *This Journal*, xcii, 196.
- ROSE, W. B., C. J. STUCKY AND L. B. MENDEL. 1930. *This Journal*, xci, 520.
- ROSENTHAL, F. AND F. PATRZEK. 1919. *Berl. Klin. Wochenschr.*, lvi, 793.
- SHERMAN, H. C., J. C. WINTERS AND V. PHILLIPS. 1919. *Journ. Biol. Chem.*, xxxix, 53.
- SHERMAN, H. C. AND S. L. SMITH. 1931. *The vitamins*. 2nd. ed., pp. 269, 276.
- SIMMONDS, N., J. E. BECKER AND E. V. MCCOLLUM. 1927. *Journ. Amer. Med. Assoc.*, lxxxviii, 1047.
- SMITH, M. I. AND E. G. HENDRICK. 1926. *U. S. Pub. Health Rept.*, xli, 201.
- SSOKOLOFF, N. A. 1924. *Deutsch. Arch. f. Klin. Med.*, cxlv, 236.
- STEENBOCK, H., E. M. NELSON AND E. B. HART. 1921. *This Journal*, lviii, 14.
- STRATHMANN-HERWEG, H. 1920. *Monatschr. f. Kinderheilk.*, xix, 20.
- STUCKY, C. J. AND W. B. ROSE. 1929. *This Journal*, lxxxix, 1.
- SURE, B., M. C. KIK AND D. J. WALKER. 1929. *Journ. Biol. Chem.*, lxxxiii, 375, 387.
1931. *Proc. Soc. Exp. Biol. Med.*, xxviii, 495.
- SURE, B., M. C. KIK AND M. E. SMITH. 1931. *Proc. Soc. Exp. Biol. Med.*, xxviii, 498.
- TERU-UCHI, Y., T. OYAMA, K. NAKAMURA AND C. WADA. 1929. *Japan. Med. World*, ix, 309.
- TILDEN, E. B. AND E. G. MILLER, JR. 1930. *Journ. Nutri.*, iii, 121.
- TURNER, R. G. 1930. *Proc. Soc. Exp. Biol. and Med.*, xxvii, 1006.
- UNDERHILL, F. P. AND L. B. MENDEL. 1928. *This Journal*, lxxxiii, 589.
- UNGLEY, C. C. AND M. M. SUZMAN. 1929. *Brain*, lii, 271.
- WHIPPLE, G. H. AND F. S. ROBSCHUIT-ROBBINS. 1930. *This Journal*, xcii, 362.
- WHITEHORN, J. C. 1924. *Journ. Biol. Chem.*, lxii, 133.
- WILLIAMS, R. R. AND R. E. WATERMAN. 1928. *Journ. Biol. Chem.*, lxxviii, 311.

- WILLIMOTT, S. G. 1928. *Biochem. Journ.*, xxii, 67.
- WILLS, L. 1931. *Brit. Med. Journ.*, i, 1059.
- WOLBACH, S. B. AND P. R. HOWE. 1925. *Proc. Soc. Exp. Biol. Med.*, xxii, 402.
1926. *Arch. Path. Lab. Med.*, i, 1.
1928. *Ibid.*, v, 239.
- WOLFF, H. G., W. P. REED AND S. COBB. 1928. *Trans. Amer. Neurol. Assoc.*, 276.
- VEDDER, E. B. AND E. CLARK. 1912. *Phillipine Journ. Sci., Sect. B*, vii, 423.
- VEDDER, E. B. 1913. *Beri-beri*. Wm. Wood & Co., New York.
- VOGT-MOLLER, P. 1931. *Biochem. Journ.*, xxv, 418.

STUDIES ON INDUCTION OF OVULATION AND THE INHIBITORY INFLUENCE OF CORPORA LUTEA ON OVULATION IN THE RABBIT

JOHN J. JARES

*From the Department of Anatomy, The University of Rochester School of Medicine and
Dentistry*

Received for publication January 21, 1932

Bellerby reported in 1929a that ovulation could be induced in rabbits by means of a single intravenous injection of an acetic acid extract of the anterior lobe of the beef hypophysis. In that year also Friedman showed that in the same species ovulation is induced by a single intravenous injection of urine from pregnant women.

The studies which are here detailed were undertaken in the hope of confirming these experiments and of applying them to the solution of certain problems of ovulation and of corpus luteum function. A preliminary report was published in the *Anatomical Record* (Jares, 1930).

1. *Repetition of Bellerby's experiment.* Fresh bovine hypophyses were obtained from the slaughter house within a few hours after death of the cattle, and the anterior lobes were shelled out. The original directions of Bellerby (1929b) for the preparation of the acetic acid extract of the anterior lobe of the hypophysis were followed. The resulting extract was clear, light yellow in color, and was non-toxic when administered intravenously in doses as large as 25 cc., but two preparations of this sort failed to induce ovulation. It was thought probable that the potency had been removed by adsorption during the filtration and kaolin treatment called for by Bellerby, and a third extract was made, this time from sheep's hypophyses, employing centrifugation instead of filtration as a means of removing solid debris, and omitting the kaolin treatment. The resulting extract was also clear and non-toxic, and induced ovulation in rabbits when given intravenously in doses of 12 cc.

2. *Ovulation induced by alkaline extract.* Typical ovulation was also induced in the rabbit by a single intravenous injection of 2.0 cc. of an alkaline extract of whole hypophyses of sheep. This extract was kindly supplied to us by Parke, Davis & Co., and was prepared by the method described by Bugbee, Simond, and Grimes (1931). The potent substance did not dialyze from this extract.

3. *Induction of ovulation by pregnancy urine.* Friedman's fine discovery was next confirmed (Jares, 1930) by administering the urine of pregnant

women to rabbits intravenously in doses of 5 cc., followed the next day by laparotomy. Ovulation was regularly found to have occurred. This phenomenon has since become well known as the basis of the now widely used rabbit ovulation test (Friedman test) for pregnancy; and even at the time of this first confirmation, it was obvious that the method could be applied to the study of many questions of reproductive physiology. Experiments were undertaken in various directions in the hope of obtaining quantitative data, and of relating the phenomenon in question to the known facts of reproductive physiology in rabbits. As a first step, to determine the minimal quantity of the untreated urine that would induce ovulation in an adult, unmated, and previously isolated rabbit, the dose administered was gradually reduced from the routine 5 cc. until a negative ovulation test was obtained. The first urine specimens that were employed in this work were obtained from a patient 25 years of age and seven and one-half months pregnant. The minimal ovulation dose was found to lie between 0.25 cc. and 0.50 cc., the former dose producing a negative ovulation test, and the latter a positive test.

4. *Measurement of inhibitory effect of corpora lutea on induction of ovulation.* At this point it became evident that here was a method whereby the supposed inhibitory influence of the corpora lutea on ovulation could be directly tested. If the corpora lutea exert an inhibitory effect upon ovulation, then the minimal dose of pregnancy urine required to produce ovulation should be larger in rabbits having functional corpora lutea in their ovaries than in rabbits containing no corpora lutea. It is evident that experiments of this sort, involving the quantitative dosage of an unknown chemical substance present in unknown concentration in the urine, must be conducted in such a way as to avoid discrepancies due to varying concentration of the urine in different samples, or to other uncontrollable influences such as pathological states of the donors. For this reason the donors were carefully selected, and each group of experiments was carried out by use of a single twenty-four hour specimen.

- In a few cases spontaneous ovulation provided the corpora lutea, and in these cases the first dose of pregnancy urine was that given to test the amount required to induce another ovulation in the presence of these corpora lutea. In most of the experiments, however, a first dose of urine was given to induce the formation of corpora lutea, and the dose to be tested was therefore the second.

Corpora lutea thus induced resemble those of experimental pseudo-pregnancy induced by sterile mating, in macroscopic and microscopic appearance, and also in duration. In all probability they are functionally equivalent to those naturally found. Winter (1931) has demonstrated that corpora lutea induced by an extract of pregnancy urine (Prolan) are able to cause changes in the uterus very similar to those occurring during

pregnancy when the normal corpora lutea are in a functional state; and Friedman (in press) has observed that corpora lutea induced by extracts of the urine of pregnancy are able to stimulate the endometrium with the production of deciduomata after traumatization, just as the naturally-occurring corpora lutea are capable of doing. At the outset of this work, exploration of the rabbits to determine the exact conditions of the follicles, ovaries, and uterus, previous to any experimentation, was performed in each experiment, but since the sexual organs in the majority of cases were in a constant and predictable condition, this preliminary control observation was dispensed with. Besides, the necessary exploratory observations made after the injections disclosed any change that had occurred previously in the follicles, as in the instances of spontaneous ovulation, mating (x-413), and hemorrhagic follicles. No difficulty whatsoever was encountered in readily recognizing the conical, bright-red, freshly ruptured follicles, and the number recorded at the laparotomy performed fifteen to thirty hours after the first injection was completely verified at the second exploration made at a later stage—usually ten days afterwards—by the large and even more easily discernible corpora lutea. Actual extrusion of the ova in these artificially-induced ovulations was proven by washing out the Fallopian tubes with normal saline twenty hours after the injection of pregnancy urine and recovering the ova under the microscope.

Repeated injections of pregnancy urine into the same rabbit cause complications and abnormal conditions, as described below, and therefore the results obtained by the first injection are considered more significant than those following the second, third, and fourth injections, and accordingly have been tabulated separately.

The first group of experiments (tables 1 and 2) was made with urine specimen C, a twenty-four hour specimen obtained from a woman 26 years of age and two and one-half months pregnant. No steps were taken to render the urine aseptic. The total volume was 1215 cc., to which were added 5.0 cc. of chloroform. The specimen was stored in the refrigerator throughout the experimental period.

Reference to table 2, "first injection" portion, shows that 0.05 cc. induced ovulation in rabbits having no corpora lutea in their ovaries, whereas 0.15 cc. was required in rabbits having functional corpora lutea, considering approximately fifty per cent of the cases. Thus a difference of 200 per cent in minimal effective quantities was obtained here. The portion of table 2 wherein all cases are recorded, including the first, second and third injections (into a single test animal) and also the doses diluted up to larger volumes with distilled water, further confirms the results obtained with the first injections recorded in the same table even though complications enter to a greater or lesser degree, and the results are probably not quite as significant as with the first injections. Diluting

the urine with distilled water appeared to reduce its potency somewhat but the findings are rather limited here.

TABLE 1
Urine specimen C—2.5 months' pregnancy stage

NUMBER OF RABBIT	INJECTION NUMBER	URINE INJECTED	DAYS BETWEEN INJECTIONS	NUMBER OF RUPTURES	NUMBER OF CORPORA LUTEA	AGE OF CORPORA LUTEA	NUMBER OF VERY LARGE FOLLICLES	NUMBER OF LARGE FOLLICLES	RUPTURES + VERY LARGE FOLLICLES
		cc.				days			
x-281	1	5.0		8			6		14
	2	0.05*	9		3†	9		11	
	3	0.10	5		3†	14	11		
x-282	1	5.0		13					13
	2	0.05*	9		13	9		9	
	3	0.15	5	9	13	14	6	4	
x-283	1	0.30		13			1		14
x-284	1	0.10		5			9		14
x-285	1	0.05		2			11		13
	2	0.05	8		2	8	14	6	
x-286	1	0.025*					13	3	13
x-287	1	0.05*					13		13
x-288	1	0.10		12			1		13
x-289	1	1.0*		12					12
	2	0.15	10	7	12	10	7	6	
	3	0.15	3	4	19	3; 13	2	4	
x-290	1	5.0		21					21
	2	0.05	10		21	10		10	
	3	0.10	3		21	13	8		
x-291	1	5.0		24					24
	2	0.05	10		24	10		14	
	3	0.15	3		24	13	18	4	
x-292	1	5.0		18					18
	2	0.10	10		18	10		14	
	3	0.15	3		18	13	14		
x-293	1	0.025					14		14
x-294	1	0.05		2			9		11
	2	0.10	8		2	8	9		
	3	0.18	4	4	2	12	4	3	
x-295	1	0.10		2			11		13
	2	0.15	8	11	2	8	6	5	
	3	0.15	4		13	4; 12	4	3	
x-296	1	0.05					12		12
	2	0.05	1				9	8	
	3	0.08	3	1			14		
x-297	1	0.10		6			7	1	13

* Diluted.

† One ovary excised.

Taken as a whole, table 2 indicates that the general run of rabbits, unselected except for sexual maturity and isolation, respond with striking similarity to a given amount of the urine both in the normal state, and under the influence of corpora lutea. It seems probable that even greater uniformity of response to a given quantity would have resulted if the urine specimen had been initially freed of certain substances which appeared later and tended to cause some difficulty in maintaining a constant concentration of the active substance in each dose. However, in all these

TABLE 2
Urine specimen C—2.5 months' pregnancy stage

FIRST INJECTION—NO CORPORA LUTEA PRESENT			FIRST INJECTION IN PRESENCE OF CORPORA LUTEA		
Urine	+	—	Urine	+	—
cc.			cc.		
0.025		1	0.05		3
0.05	2	1	0.10		2
0.10	4		0.15	2	
0.30	1				
5.0	5				

All cases, including first, second and third injections and dilutions

WITHOUT CORPORA LUTEA			WITH CORPORA LUTEA		
Urine	+	—	Urine	+	—
cc.			cc.		
0.025	0	2	0.05		5
0.05	2	3	0.10		4
0.08	1		0.15	4	3
0.10	4		0.18	1	
0.30	1				
1.0	1				
5.0	5				

experiments, the urine specimen was adequately agitated immediately before each injection.

About thirty days after the urine specimen had been voided, the minimal ovulation dose under normal conditions increased progressively with the age of the urine, due to, apparently, the deterioration of the active substance. The observations made under the latter conditions are not recorded here. At the onset of deterioration a good working knowledge of the active amounts under the various conditions had been gained, and just when more data on the critical dosages were in order, observations were forced to halt.

A second group of experiments (tables 3 and 4) was performed with

TABLE 3
Urine specimen J—2 months' pregnancy stage

NUMBER OF RABBIT	INJECTION NUMBER	URINE INJECTED	DAYS BETWEEN INJECTIONS	NUMBER OF RUPTURES	NUMBER OF CORPORA LUTEA	AGE OF CORPORA LUTEA	NUMBER OF VERY LARGE FOLLICLES	NUMBER OF LARGE FOLLICLES
		cc.				days		
x-401	1	0.10		3	1*	10 (?)	14	
	2	0.30	11		3	11		19
	3	0.50	3		3	14	13	10
	4	0.60	1		3	15	24	
x-402	1	0.05						16
	2	0.08	1					13
	3	0.12	1				13	
	4	0.15	13	3			7	
x-403	1	0.025						13
	2	0.08	1					12
	3	0.15	1				4	10
	4	0.25	13	2	1†	13 (?)	5	1
x-404	1	0.10						12
	2	0.10	27					14
x-405	1	0.15		13				1
	2	0.50	11	18	13	11		2
	3	0.35	10	5	18	10		14
x-406	1	0.12		10			4	
	2	0.40	11	16	12	11		3
	3	0.30	10	7	16	10		11
x-407	1	0.10					17	4
	2	0.15	1				22	
	3	0.20	9	4			14	
x-408†	1	0.12						13
	2	0.18	1				11	
	3	0.15	9					15
x-409	1	0.10						10
	2	0.15	1					10
	3	0.15	9					12
x-410	1	0.15		4			6	2
	2	0.30	12	5	4	12	1	9
x-411	1	0.12		12			1	1
	2	0.35	11	11	12	11		
x-412	1	0.10						7
	2	0.20	1	2			6	3
x-413	1	0.40		7	9§	17		6
x-414	1	0.12					9	
	2	0.15	9	4				6
x-415	1	0.12		11				5
	2	0.25	9	6	11	9		6

* Resulting from a spontaneous ovulation.

† Probably a luteinized follicle with contained ovum.

‡ Very large animal.

§ Resulting from a normal mating.

TABLE 3—*Concluded*

NUMBER OF RABBIT	INJECTION NUMBER	URINE INJECTED	DAYS BETWEEN INJECTIONS	NUMBER OF RUPTURES	NUMBER OF CORPORA LUTEA	AGE OF CORPORA LUTEA	NUMBER OF VERY LARGE FOLLICLES	NUMBER OF LARGE FOLLICLES
		cc.				days		
x-416	1	0.15		8				
	2	0.20	10	6	8	10		5
x-417	1	0.12		1			1	5
	2	0.15	8	8	1	8		1
x-418	1	0.15			9*	10 (?)		13
x-419	1	10.0		17				
	2	0.20	8	10	17	8	1	
x-420	1	5.0		11				
	2	0.15	9		11	9		11

urine specimen J, a twenty-four hour specimen obtained from a woman 18 years of age and two months pregnant. The total volume of the specimen was 1150 cc., to which were added 5.0 cc. of chloroform. The specimen was stored in the refrigerator throughout the experimental period. This specimen was tested for oestrin in spayed rats and proved negative. The difference in the minimal ovulation dose in rabbits having no corpora lutea and in those having functional corpora lutea was not as great with this specimen as with specimen C, being 0.12 cc. and 0.20 cc. (first injections, table 4), respectively, a difference of 67 per cent, considering approximately 50 per cent of the cases. As with specimen C, deterioration began in about thirty days, the minimal ovulation dose increasing and the results becoming somewhat inconsistent. The observations made after the specimen reached thirty days of age are not reported here. Taken as a whole, the data observed with specimen J were not as consistent among themselves as those of specimen C, nor were the differences under the various experimental conditions as great and as definite as with specimen C. This can be explained, partially at least, by greater differences in weight, age and other details than happened to be the case with the animals used with specimen C. Two cases (x-401 and x-417) in which ovulation was induced in the presence of a single corpus luteum with doses smaller than those found necessary to induce ovulation in rabbits having the usual number of corpora lutea, are not included in the summary table (table 4), since there were special circumstances which made them not comparable with the others.

These experiments indicate that in the presence of corpora lutea about three times as much of the pregnancy urine is required to produce ovulation as in the absence of corpora lutea. The most obvious interpretation of this finding is that the corpora lutea exert an inhibitory effect upon

action of the ovulation-inducing hormone. Before accepting this explanation, however, two other possible explanations must be considered.

The possibility that an immunization was produced by an injection of the urine specimen, thereby accounting for the larger amount required to induce ovulation under the lutein condition, was eliminated experimentally: Rabbit x-414 will serve as an example. An initial injection of

TABLE 4
Urine specimen J—2 months' pregnancy stage

FIRST INJECTION—NO CORPORA LUTEA PRESENT			FIRST INJECTION IN PRESENCE OF CORPORA LUTEA		
Urine	+	—	Urine	+	—
cc.			cc.		
0.025		1	0.15		2
0.05		1	0.20	2	
0.10		4	0.25	1	
0.12	4	2	0.30	1	
0.15	3		0.35	1	
5.0	1		0.40	2	
10.0	1		0.50	1	

All cases, including first, second, third and fourth injections

WITHOUT CORPORA LUTEA			WITH CORPORA LUTEA		
Urine	+	—	Urine	+	—
cc.			cc.		
0.025		1	0.15		2
0.05		1	0.20	2	
0.08		2	0.25	2	
0.10		5	0.30	2	
0.12	4	3	0.35	2	
0.15	5	5	0.40	2	
0.18		1	0.50	1	1*
0.20	2		0.60		1*
5.0	1				
10.0	1				

* x-401, see text.

0.12 cc. of specimen J was insufficient in this case to cause ovulation. A second injection of 0.15 cc., nine days after the first, induced ruptures. Furthermore, the massive dose of 10.0 cc. of specimen J was administered to rabbit x-419 resulting in seventeen ruptures, and eight days later, a dose of 0.20 cc. induced a typical ovulation, ten ruptures resulting.

A second possible explanation requires attention. Under the column headed "ruptures plus very large follicles," table 1, there is noted a fairly

constant value, averaging thirteen, except for three cases where large doses of urine were administered and from eighteen to twenty-four ruptures were produced. Evidently, with these large doses, all the follicles attaining even a small macroscopical size are forced to rupture. It might logically be argued therefore that a normally effective dose, administered shortly after a huge one, would find follicular conditions opposed to immediate rupture, and thus fail to induce ovulation. This could be a possible explanation for cases x-290, x-291, and x-292, in which 5.0 cc. were administered in each instance in order to form corpora lutea, and 21, 24, and 18 follicles, respectively, were ruptured. Ten days later, 0.05 cc., 0.05 cc., and 0.10 cc., respectively, was injected, and no ruptures were found in any instance at exploration the following day. However, such conditions could not have obtained, apparently, in cases x-285 and x-294, in which 0.05 cc. was administered at the very first injection, and only two ruptures occurred in both instances, thereby leaving numerous very large follicles for the second test quantities—0.05 cc. and 0.10 cc. respectively—which did not induce ovulation. It should be pointed out here that two separate doses, subminimal for a given animal, were not summated to induce follicular rupture, regardless of the interval—22 hours and upward—between the administrations. Such a case is exemplified by rabbit x-296, whose ovaries contained many very large follicles but no ruptures as a result of the very first injection of 0.05 cc. A second injection of 0.05 cc. administered one day after the first, served to further increase the size of some of the follicles, but produced no ruptures. The sizes of the follicles are not expressed in terms of exact unit diameter, but more generally, since it has been observed that relatively small follicles in one rabbit and relatively large follicles in another, will both rupture in response to the same quantity of active substance.

With both the alternative explanations thus ruled out, it seems probable than an inhibitory effect of the corpora lutea is the actual explanation of the result obtained in these experiments. It was observed in numerous cases that no correlation existed between the number of corpora lutea in an ovary and the number of follicles induced to rupture by the urine injection. When there was a great disparity between the number of corpora lutea in the two ovaries of an animal, the number of newly ruptured follicles in each ovary tended toward equality. It seems therefore that the inhibitory effect upon ovulation exerted by the corpora lutea, acts upon both ovaries, presumably by a humoral mechanism, and is not due to merely local effects.

5. *Threshold effect.* With the just effective minimal dose the number of ruptures is lowest, while the number of enlarged follicles is generally correspondingly high (table 1), indicating that a certain threshold amount of the stimulating substance is necessary for actual rupture to occur in

any one case. The reverse was observed in those cases where the largest quantities of urine were employed, the number of ruptures being high, and the follicles being few and small. Intermediate doses produced corresponding events, and, in general, the number of ruptures was proportional to the dose up to a certain limit, above which still larger doses did not increase the number of ruptures. Since the above problems are of a statistical nature, and since the data are too few, no graphical representations are herewith attempted.

6. *Effect of dosage on the time required for induction of ovulation.* In an effort to accelerate the ovulatory process by means of a huge dose of pregnancy urine the following experiment was carried out: Rabbit x-219 received 0.38 cc. of pregnancy urine (4 months stage) intravenously. Rabbit x-233 received 5.0 cc. of the same urine specimen at the same time. Both animals were anesthetized with urethane and explored simultaneously. Care was exercised not to touch the ovaries and warm saline swabs were placed on the exposed tissue during the very brief observational exposures. Actual follicular rupture began in both animals nine hours after injection. At the end of the tenth hour the animal receiving the small dose possessed a total of ten ruptures, 5 large follicles, and three fairly dark blood follicles. At the twelfth hour no further changes could be observed, except that the bright-red orificial plugs were larger and more distinct and the conical shape of the ruptured follicle was more pronounced. The rabbit receiving the large dose possessed a total of five ruptures, and twelve very large follicles at the tenth hour; at the twelfth hour, fifteen ruptures were observed. It is thus inferred that the larger amount of stimulating substance probably produced more widespread follicular development and secretion and more ruptures than the small amount, but that the effective reaction time for rupture was approximately the same in both cases.

7. *Ovulation induced during pregnancy.* A single observation of induction in a pregnant rabbit by administration of pregnancy urine was made. Rabbit x-413 (table 3) was injected with 0.40 cc. of specimen J on the seventeenth day following mating. Exploration on the eighteenth day disclosed seven ruptures, nine whitish-yellow corpora lutea resulting from the mating, and nine normally developing fetuses. Resorption of the fetuses occurred later, but no inference can be made on a single case as to the significance of this fact.

The observation that the mating of a rabbit during any stage of pregnancy or pseudo-pregnancy does not effect an ovulation (Hammond and Marshall, 1925), while administration of the gonad-stimulating substance induces follicular rupture, suggests that some controlling factor is present in the normal animal. This factor appears to be the inhibitory influence of the corpora lutea, which could effect this result by either depressing the production and secretion of the active substance, or by inhibiting its

functional activity in the follicles. It is possible that the amount of stimulating substance as administered experimentally is too great to be antagonized or that optimal opportunity is not afforded the corpora lutea when the substance is introduced directly into the circulation.

8. *Repeated induction of ovulation at short intervals.* Ovulation could be induced repeatedly (x-289, x-405, x-406), and whenever desired, by successive injections of pregnancy urine, providing the dose was large enough. By such means rabbits have been kept in a condition of excessive luteinization for as long as seven weeks, at which time experimentation was discontinued. The ovaries became greatly enlarged and monstrously shaped after the first few ovulations, the corpora lutea bulging and crowding in every direction.

9. *Experimental production of hemorrhagic follicles.* Hemorrhagic follicles were found present at the majority of the exploratory observations. In the case of rabbit x-297, forty blood follicles were found in the two ovaries at the exploration following a first injection of 0.10 cc. of specimen C. No observation as to the number of blood follicles previous to the above injection was made, but their small size and dark appearance indicated that they had been present for some time prior to the above experimentation. However, in other cases, the large, distended, bright-red blood follicles, observed fifteen to thirty hours after the first injection indicated that hemorrhagic follicles may result from administration of the gonad-stimulating substance. Only a few days and two or three doses are required for such a follicle under this forced stimulation to turn from a bright-red color, through red-black, blue-black, and black, and to assume relatively gigantic proportions. A good example of such blood follicles was observed in x-401. These blood follicles occur normally in the rabbit and have been described by Hammond and Marshall (1925). Their similar occurrence under these experimental conditions may possibly indicate that the same processes are involved as under normal environmental conditions, except that the abnormal process as a whole is speeded up under adequate, experimentally-forced stimulation as used here. Those enlarged follicles having only a very slightly bright-red color have been included in the tables as very large follicles and large follicles, depending on their size. However, it should be parenthetically mentioned here that even a recent, true blood follicle was never observed to have ruptured.

10. *Observation of the actual rupture of Graafian follicles.* Actual rupturing of the follicle was observed many times during the above experimentation. The phenomena occurring during the process of ovulation in the rabbit as observed in these experiments confirm the pioneer studies of Walton and Hammond (1928). In several instances ruptures occurred during exploratory observation as long as seventeen to twenty-six hours after injection. It was also noted during an ovariectomy performed

twenty-six hours after a large injection of pregnancy urine that even gentle handling of the ovary caused several very large follicles to rupture. However, in the above tabulated cases, touching or handling of the ovary itself was rigidly avoided.

DISCUSSION. It is interesting to note that Hammond in 1925 postulated that "the number of follicles which ripen depends upon a limiting amount of *some nutritive substratum* in the blood supply (Heape's 'generative ferment') rather than on any inherent potentiality of the ovary itself," and together with Walton (1928) showed that artificial rupture or ablation of ripe follicles is followed by an immediate compensatory growth of new follicles. The experimental findings recorded above, obtained by quite different methods, bear out and extend these contentions, since follicular growth, maturation and rupture were repeatedly induced at short intervals by the gonad-stimulating substance.

The difference in the minimal ovulation amounts of pregnancy urine in animals with and without corpora lutea appears to lend support to the theory that the corpora lutea inhibit ovulation. However, further general knowledge concerning effective amounts under more varied conditions, and more minute study of the mechanism of the functional activity of the stimulating substance, and also of the inhibiting substance seems necessary before a final conclusion can be made.

Much conjecture and evidence has accumulated regarding the inhibitory influence of corpora lutea on ovulation. However, little of it seems to be free from complications and interfering processes both normal and abnormal. Recently, Patel (1930) has produced evidence that the administration of corpus luteum hormone caused a reduced reactivity of the ovaries of immature mice to the gonad-stimulating substance derived from human placenta, and also probably inhibited the secretion of this potent substance by acting on the pituitary. Wolfe (1931) showed that a much larger amount of the sow's anterior hypophysis tissue in saline suspension was required to induce ovulation in the rabbit when the ovaries of the donors contained active corpora lutea.

This recent, more direct evidence, together with the well-known phenomenon that the squeezing out of the cow's corpora lutea hastens the next oestrous period and ovulation, supports the data reported here, and seems to indicate that there is an antagonism between the corpus luteum hormone and the gonad-stimulating substance or substances of the anterior hypophysis or the similarly acting substance in pregnancy urine.

SUMMARY

1. Ovulation has been induced in the rabbit by a single intravenous injection of the following: 1, an acid extract of the anterior hypophysis (confirming Bellerby); 2, an alkaline extract (Parke, Davis) of the anterior hypophysis, and 3, human urine of pregnancy (confirming Friedman).

2. The minimum ovulation dose (considering approximately 50 per cent of the cases) of untreated human pregnancy urine in normal rabbits varied with the length of gestation of the donor and with the donor as an individual: 0.05, 0.12 and between 0.25 and 0.50 cc. were required from gestational stages of approximately 2.5, 2.0, and 6.5 months, respectively.

3. The minimal dose of untreated pregnancy urine necessary to induce ovulation (considering approximately 50 per cent of the cases) was found to be definitely larger in rabbits whose ovaries contained active corpora lutea. These corpora lutea were experimentally produced by various quantities of untreated urine from the first two human donors mentioned above. One spontaneous ovulation and one fertile mating accounted for the corpora lutea in two additional cases. The minimal ovulation dose in rabbits having corpora lutea was raised from 0.05 to 0.15 cc., a difference of 200 per cent, (employing one preserved 24-hour urine specimen throughout the experimentation), from 0.12 to 0.20 cc., a difference of 67 per cent, (using a single 24-hour specimen).

4. Ovulation in the rabbit has been directly observed. The follicular changes noted in these experiments before, during, and after actual rupture and extravasation of the ovum confirm Walton and Hammond's observations.

5. A massive dose of pregnancy urine does not hasten the rupturing time of about ten hours after administration.

6. Ovulation was induced in a rabbit on the seventeenth day of gestation by injection of pregnancy urine.

7. Changes in the follicle under these experimental conditions appear to be similar to those occurring normally. Hemorrhagic follicles were observed to occur as a result of the administration of anterior hypophyseal substance, and both their number and size were dependent upon the frequency and magnitude of the forced stimulation.

I am greatly indebted to Prof. Karl M. Wilson and the Department of Obstetrics and Gynecology of Strong Memorial Hospital for excellent coöperation in providing the urine specimens and information regarding the donors.

It is a pleasure to acknowledge the interest and assistance of Prof. George W. Corner in these investigations.

BIBLIOGRAPHY

- BELLERBY, C. W. 1929a. *Journ. Physiol.*, lxxvii, 33.
1929b. *Journ. Physiol.*, lxxvii, 32.
BUGBEE, E. P., A. E. SIMOND AND H. M. GRIMES. 1932. *Endocrinol.*, xv, 41.
FRIEDMAN, M. H. 1929. *This Journal*, xc, 617.
1932. *Journ. Pharm. Exper. Therap.* (in press).
HAMMOND, J. AND F. H. A. MARSHALL. 1925. *Reproduction in the rabbit*. London.

- JARES, J. J. 1930. Anat. Rec., xlv, 264.
PATEL, J. S. 1930. Quart. Journ. Exper. Physiol., xx, 245.
WALTON, A. AND J. HAMMOND. 1928. Brit. Journ. Exper. Biol., vi, 190.
WINTER, E. W. 1931. Zeitschr. f. Geburtshülfe und Gynäkol., ci (1), 196.
WOLFE, J. M. 1931. Amer. Journ. Anat., xlviii, 391.

ON THE EFFECTS OF POLARIZATION OF NERVE FIBERS BY EXTRINSIC ACTION POTENTIALS¹

E. A. BLAIR AND JOSEPH ERLANGER

*From the Physiological Department, Washington University School of Medicine,
Saint Louis*

Received for publication May 12, 1932

There are several hypotheses assigning important physiological rôles to currents originating in nerve. Among others may be mentioned the theory that attributes impulse propagation to restimulation by an eddying along the fiber of the nerve's action current; the view that Wedenski block is a state of postcathodal depression determined by the potential of the blocked nerve impulse (Erlanger and Blair, 1931b); and the view attributing the relatively refractory phase to the negative phase of the irritability effect determined by the action potential of nerve (Erlanger and Blair, 1931a). These hypotheses predicate action of the nerve's potential on the fiber in which it originates. As instances of suggested extraneous actions of nerve potentials may be mentioned Adrian's view (1930) attributing spontaneous repetitive responses of the fibers of a cut nerve to stimulation by the nerve's demarcation potential, and so-called rheoscopic stimulation.

Our study of the summation and depression intervals determined by brief subthreshold shocks (Erlanger and Blair, 1931a) suggested the idea that such current as leaks from active fibers of a nerve and enters adjacent fibers that happen to be inactive locally must have the effect, just as does a subthreshold shock, of first increasing the fibers' irritability and then decreasing it. In so far as this occurs there must be produced a state tending to bring into phase impulses running slightly out of phase in neighboring fibers conducting at the same rate; it would constitute a mechanism making for the synchronization of action potentials running out of phase in homogeneous fibers of a nerve. It was to ascertain whether there is any such action in normal nerve that this investigation was undertaken. Our results in this respect, however, have proven entirely negative. The experiments will, therefore, be considered in the briefest possible manner.

The *methods* employed involve registration by the cathode ray oscillograph of the amplified action potentials as described in previous publications from our laboratory, except that in our present plant the von Ardenne

¹ This work was made possible in part by assistance from a grant made by the Rockefeller Foundation to Washington University for research in science.

oscillograph² replaces that of Johnson. The deflections on the screen of this tube may be made so luminous that they can be directly photographed on sensitive film through a quick lens. The pictures thus obtained are much sharper than our previous contact prints. At the higher driving voltages the von Ardenne tube has about one-fifth the sensitivity of the Johnson tube. To overcome this difference amplification has been more than correspondingly increased. A more complete description of our methods will be given elsewhere.

RESULTS. Mention may be made first of an experiment performed to ascertain whether there is any synchronizing action in normal nerve. The phrenic nerve of the dog is removed from its origin in the neck down to the diaphragm. Two of its roots of origin are placed one each on the terminals

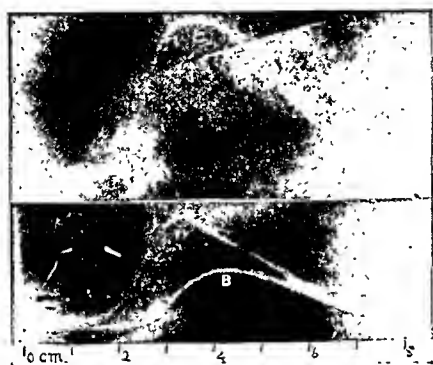


Fig. 1. Submaximal alpha action potentials of the dog's phrenic, each started by stimulation of a different trunk of origin. Conduction 15 cm. *A* and *B* are separate records printed in superposition. *C* is the record of these two action potentials travelling simultaneously but out of phase. Reduced.

of an inductorium and arrangements are made to lead monophasically from the distal end 15 cm. away the action potentials resulting from stimulation of the roots. First, records are made of action potentials slightly above threshold in amplitude from each of the roots separately (see fig. 1). The shocks are so timed that the action potentials they initiate reach the lead at different but known intervals after the spot has started on its horizontal sweep across the screen of the tube. Then the two roots are stimulated during one and the same sweep with some one pair of these stimulation intervals.

It has been found in such experiments that the figure derived from the algebraic sum of the two action potentials running independently invariably is superimposable on the record of the two comparable action potentials running simultaneously but slightly out of phase as summed by the record-

² Obtained through the General Radio Company, Cambridge, Massachusetts.

ing mechanism, when an amplitude correction is applied that is necessitated by the characteristic curve of the amplifier (see fig. 1). This temporal agreement of the two sums has obtained no matter what have been the relations of the two action potentials to each other. Since the phrenic roots presumably contain the same kinds of axons and since these presumably are intimately mixed in the nerve trunk the conditions provided in this experiment are regarded as optimal for the manifestation of any extrinsic action fiber potentials may have in normal nerve. Comparable preparations consisting of the sciatic nerve of the bullfrog with two of its plexus trunks likewise have given negative results.

In order to give the leaking currents every possible chance to induce a response the above procedure was repeated on nerves whose reactivity was raised locally by various devices. First it was shown, for purposes of control, that a nerve whose local reactivity is rising at the cathode during the initial stages of the make of a just subthreshold constant current or of a current rising practically linearly at less than the liminal rate does not,

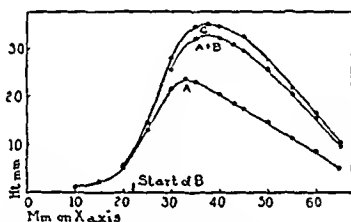


Fig. 2. $A + B$ is the sum of A and B of figure 1; C is C of figure 1. C and $A + B$ differ from each other in respect to height only.

within the limit of error, conduct more rapidly than a normal nerve. But even under these conditions it has been impossible to demonstrate by methods illustrated in figure 1 any influence of the action potentials running in one group of fibers upon the propagation rate of the action potentials running slightly out of phase with them in another group. We conclude, therefore, that *conduction* in a fiber, whether normally or hypernormally irritable, is wholly uninfluenced by currents emanating from its neighbors.

Having failed to demonstrate any effect of extrinsic nerve currents on conductivity we next planned experiments to ascertain whether the current from active fibers affects the *irritability* of neighboring inactive fibers. With our methods it is a simple matter to place an induction shock in any desired phase of an action potential moving along a nerve (Erlanger, Bishop and Gasser, 1926). In tests carried out in this way we have failed to find any enhancement of irritability of inactive fibers exposed to a passing action potential; a just subthreshold testing shock remains so in all phases of a passing submaximal action potential. Even when the irritability of the nerve is raised locally by the procedures mentioned above there is no

stimulation of additional fibers; the current escaping from active axons does not suffice to stimulate neighboring fibers even when they are about ready to fire off.

Neither is the response of a nerve to a just threshold constant current increased by leading into it, through the cathode of the polarizing current, a properly directed maximum monophasic action potential from another nerve; it is not increased even when this action potential arrives at the time the irritability of the nerve being thus polarized is at its maximum.

At this place it may be well to call attention to the fact that when nerve, which is being treated with a just subthreshold constant current, attains the level of maximum irritability and is about to respond an added shock still must be given an appreciable, even though small, intensity if it is to stimulate (Erlanger and Blair, 1931b). Failure of an applied extrinsic action potential to stimulate nerve in that state must, therefore, mean that the stimulating value of the action potential applied is less than that of the shock that is just threshold under similar circumstances. In experiments of this type the leads from the nerves supplying the extrinsic action potential were separated by a distance, about 2 cm., such that they would subtend a very large part of the nerve's potential. The voltage thus derived was not measured. If, however, it be assumed that it was 0.01 volt and that the resistance through which it acted was 100,000 ohms the current applied would have been 0.1 microampere. Under comparable conditions we have found that a rectangular constant current reaches threshold at something less than 0.5 microampere. Since a rectangular current presumably has a higher stimulating value than a current of the shape of an action potential it is fair to conclude that the stimulating values of the extrinsic action potentials have been considerably below that of the threshold constant current. Undoubtedly it is this difference that accounts for the ineffectiveness of the former in these experiments.

In the experiments just described the polarizing leads were on intact nerve surface. The results, as has been said, were negative. Since rheoscopic preparations are known to give positive results experiments were then performed in which the conditions usually obtaining in such preparations were supplied. The method used is illustrated in figure 3. The preparation consisted of two nerves. First, a constant current was found that was slightly above the threshold of both of the nerves, *A* and *B*. With the arrangement shown in the figure, where the electrodes polarizing *B* were both on intact nerve, the very much submaximal action potential determined in *B* by closure of the constant current was not increased in amplitude by leading the maximum action potential of *A* into *B* even at the moment when the irritability of the latter, as a result of the make of the constant current, was at its maximum. This part of the experiment confirms our previous experience relative to the ineffectiveness of extrinsic

action potentials when applied to intact nerve. Then *B* was killed at *C*. Now the addition of the action potential of *A* materially increased the amplitude of the action potential started in *B* by the constant current.

It has also been found that positive results are obtained when one of the terminals connecting the extrinsic potential source with the nerve rests on cut branches. With arrangements such as are shown in figure 4, in which the cathode of the polarizing current rests on cut branches, a sub-maximal action potential started at *A* and recorded at *B* increases in height slightly when the action potential is made to pass *C* at the time the irritability there is rising as a result of the prior closure of the constant current; the effect is maximal at the time the irritability rise due to the closure is at its maximum.

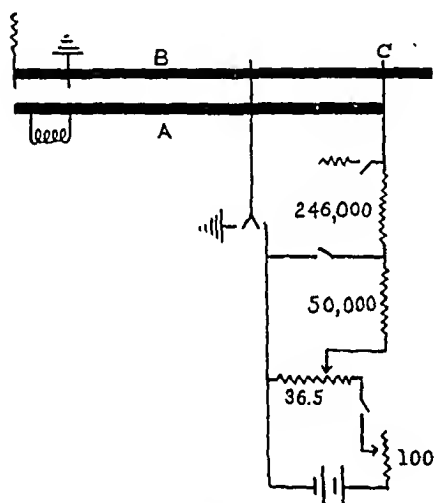


Fig. 3

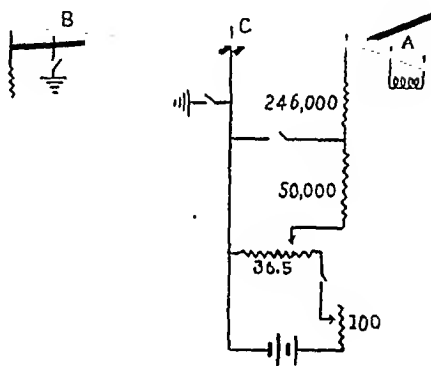


Fig. 4

Fig. 3. Arrangements employed to demonstrate the effect of extrinsic action potentials from one nerve on another nerve, either intact or injured.

Fig. 4. Arrangements employed to demonstrate the stimulating effect of the potentials from one set of fibers upon another at cut branches.

The rôle of the cut end in making effective the current determined by the applied action potential has not been investigated, since we were concerned with the effects developed by action potentials in normal nerve only. It is to be presumed, however, that an important factor in cut nerve is the influence of the open connective tissue and myelin sheaths on the distribution of the current lines determined by the action potential.

The two types of experiments last described were devised as a last resort, for we had reached the point where we began to feel that we could not repeat a simple rheoscopic experiment. Now, however, that we have obtained these positive results we feel justified in concluding that by our

negative experiments we have demonstrated that such current as leaks from fiber to fiber in normal nerve is without physiological significance.

BIBLIOGRAPHY

- ADRIAN, E. D. 1930. Proc. Roy. Soc. B, cvi, 596.
ERLANGER, J., G. H. BISHOP AND H. S. GASSER. 1926. This Journal, lxxviii, 537. .
ERLANGER, J. AND E. A. BLAIR. 1931a. This Journal, xcix, 108.
1931b. This Journal, xcix, 129.

THE RELATIVE ABSORPTION RATES OF DEXTROSE AND LEVULOSE¹

G. E. BURGET, PHILIP MOORE AND ROBERT LLOYD

From the Department of Physiology, University of Oregon Medical School

Received for publication March 2, 1932

During a series of studies on closed intestinal loops in dogs it was found that these isolated segments of small intestine with intact mesentery, under favorable conditions became normal in gross and histologic appearance. Preliminary experiments also showed that the loop while in this apparently normal state absorbed quite readily substances injected into it. In order to further test the reliability of the chronic closed loop for absorption studies a series of experiments has been carried out using three methods: *a*, the closed intestinal loop of dogs; *b*, loops of small intestine of anesthetized rabbits; *c*, the gastro-intestinal tract of normal rats. The following report is concerned with the absorption rates of dextrose and levulose. Pfanstiehl sugars with specific rotatory powers were used throughout.

PROCEDURES. *a. Closed loop of small intestine in dogs.* About two weeks after the isolated loop has been made, barring accidents, the animal should be in good condition (1). The loop will have quieted down and the closed ends completely healed. If no fluid is being held in the loop it may be considered to be in normal condition. The skin over the site of the loop attachment to the abdominal wall is cleansed with alcohol and a sterile needle (preferably no. 19, 1.5 inch long) is inserted into the loop. If it has not been washed out for a few days, it should now be washed by gently injecting and aspirating warm physiological salt solution. A loop approximately 12 cm. long may be given 10 cc. of a 20 per cent solution of sugar. One hour after giving the sugar, the loop is aspirated and washed with warm physiological salt solution three times to remove that not absorbed. The total amount of sugar removed is then determined by the Shaffer-Hartman method and the amount of absorption calculated.

b. Loops of small intestine of anesthetized rabbits. Young rabbits weighing five to six pounds having been without food for twenty-four hours were anesthetized and the abdomen opened. A section of the ileum about 90 cm. long was ligated at each end and two ligatures about one-half inch apart were placed in the middle of the segment. The sugar solutions were in-

¹ This work was supported in part by Grant 191 of the Committee on Scientific Research of the American Medical Association.

jected with a hypodermic needle and the abdomen closed. The operation was carried out as quickly as possible (approximately five minutes) in order to minimize the length of time the animal remained under the anesthetic. Heat from an electric reflector was then applied and in twenty to thirty minutes the animal appeared to have recovered from the anesthetic. At the end of one hour it was killed by a blow just back of the head. The segments were removed and the contents emptied into beakers. The mucosa was carefully washed with 75 cc. of hot water to remove final traces of unabsorbed sugar.

c. *The rat method* used by Cori (2) was carried out in most respects as indicated in his work. The last food was given forty-eight hours before the experiment but water was left in the cage during this period. The cages had screen bottoms to allow feces to drop out of reach of the animals. The weights of the rats varied between 160 and 310 grams. By means of a no. 8 French catheter 2 cc. of a 50 per cent solution of the sugar were introduced into the stomach. After the injection the amount of sugar remaining in the catheter was determined. At the end of the absorption period the animal was killed by a blow on the head. The abdomen was immediately opened and ligatures placed around the esophagus just above the cardia and around the ileum near the ileo-colic valve. It was found unnecessary to include the lower bowel. After removal, this part of the gastro-intestinal tract was thoroughly washed by forcing 75 cc. of warm water through it in three separate portions. After making the fluid content and washings up to 100 cc. the amount of sugar was determined.

The amount of the sugar absorbed was arrived at by accurately estimating the amount placed in the intestinal tract or segment thereof and subtracting from this the amount of sugar recovered. Normax glassware checked against United States Bureau of Standards certified burettes was used entirely. The sugar solutions were made by weight and after twenty-four hours checked in the polariscope. In the dogs and rabbits the solutions were introduced with a syringe after being measured out with accurate pipettes. The amount of sugar remaining in the syringe was determined on several occasions and found to be less than 30 mgm. Since this amount was uniform for the two sugars it was left out of the calculations. In rats where a small catheter was used the amount of sugar remaining in and sticking to the outside of the catheter was determined in each case. Where this amount was above 50 mgm. it was subtracted from the estimated amount given. In all experiments the total amount of sugar recovered was diluted to 100 cc. in a volumetric flask and a one to fifty dilution was made of this for Shaffer-Hartman determination. Further care was taken to adjust the dilutions when indicated to bring the final reading in cubic centimeters of thiosulphate used for titration within the limits of 12 to 18 cc. since this has been shown to be the amount of sugar most accurately determined by

this method. The amount of protein in the recovered specimens was found to be a negligible factor in the sugar estimations. A study of the Shaffer-Hartman method on known dilutions of levulose has shown that the method gives results 10 per cent too low. This has also been taken into account in the levulose determinations.

The amounts of absorption of levulose and dextrose in one hour when 10 cc. of a 20 per cent solution (2 grams) were injected into closed loops of dogs were studied. The average of 19 separate experiments on eight different dogs was 0.86 gram for dextrose; 26 experiments on eight different dogs using levulose was 0.79 gram. In these experiments as in those to follow, it is considered that the loops were approximately at the same level in the ileum and near the same size. While some small differences may have existed, the use of the animal for both sugars would tend to exclude these as factors that would alter the conclusions.

TABLE I

Results of repeated experiments on individual dogs expressed as grams of sugar absorbed by closed loops in one hour from 10 cc. of a 20 per cent solution of the sugar

dog 61		dog 132		dog 135		dog 131	
Levulose	Dextrose	Levulose	Dextrose	Levulose	Dextrose	Levulose	Dextrose
1.15	0.98	0.72	1.38	0.90	1.03	0.61	1.01
0.81	0.97	0.81	1.11	0.76	0.97	0.63	0.97
0.86	0.99	0.86	0.74	0.71	0.72	0.60	0.85
		0.93	0.75	0.70	0.73	0.67	0.77
		0.66	0.65			0.60	0.93
						0.59	
						0.56	
0.95	0.98	0.80	0.91	0.77	0.86	0.61	0.90

When 10 cc. of a 10 per cent solution of sugar was given the average absorption for dextrose (5 expts.) was 0.52 gram and for levulose (12 expts.) 0.46 gram. Another series of seven experiments with each sugar (20 cc. of a 10 per cent solution) gave an average of 0.91 gram for dextrose and 0.95 gram for levulose.

The best comparison of absorption rates would obtain where a series of experiments is carried out on a given dog alternating the sugars as the series progresses. Any changes in the loop or in the condition of the dog is thus reflected equally upon the two sugars. In table 1 are shown the results from experiments on four dogs. Dog 131 seemed to show a distinct preference for dextrose while the other animals showed a tendency to absorb dextrose only slightly more rapidly.

The first series of rat experiments was carried out upon rats varying in

weight from 160 to 310 grams. These were all healthy animals but obtained from different sources, consequently their previous diet may have varied. Periods of 30, 60, 90 and 120 minutes were used. The average absorption of levulose in each of these intervals was less than that of dextrose. The greatest difference was in the two-hour period and amounted to 19 per cent. However, in view of a seemingly consistently slower rate for levulose absorption, it was decided to run a longer series on a group of rats of the same stock that had been kept on a uniform, adequate diet. These were all young rats with weights varying from 185 to 300 grams.

In this series twenty-one rats were given levulose and twenty-four dextrose. The absorption period was one hour. The greatest amount of levulose absorbed was 0.70 gram; the least 0.41 gram. The greatest amount of dextrose absorbed was 0.80 gram while the least amount was 0.35 gram. The average amount of levulose absorbed per rat was 0.55 gram; and of dextrose, 0.57 gram. It would seem that if any marked difference in the rates of absorption of these sugars by rats exists, these experiments would have shown it.

In 36 rabbit experiments using an absorption period of one hour the above results were confirmed. This method is less desirable because of the effects of the anesthesia than either of the other methods. When 5 cc. of a 10 per cent solution were given the average absorption was 0.29 gram for dextrose and 0.28 for levulose. Further results were obtained on giving 1 gram of the sugar (some were given 10 cc. of a 10 per cent solution and others 20 cc. of a 5 per cent solution). The average absorption was 0.46 gram dextrose and 0.44 gram levulose. The sugars were alternated with respect to "high" and "low" positions in the ileum and the averages of the lengths of loops used for the two sugars were approximately equal.

Discussion. Different investigators have reported that levulose has a slower absorption rate than dextrose (3), (4). Their work has hardly been carried out under conditions that would permit of definite conclusions. The more recent work of Cori where rats were used would seem less open to criticism. He found that if the absorption rate of dextrose be taken as 100, that of levulose is 43. This remarkable difference was explained by Cori on the basis of stereoisomerism. Our results show no such difference in any of the methods used. There is considerable variation in the amounts of absorption in all series. This has been the experience of other investigators. Cori drew his conclusions on the amount of absorption of sugar the first hour by averaging the results of five experiments. In the case of levulose the maximum and minimum amounts of absorption varied by 38 per cent, yet the average of these was carried through for estimation of absorption during the second, third, and fourth hours in different groups of rats. Only by increasing the number of rats in a series could this obvious possible source of error be eliminated. From a series

of 37 rats given levulose we found the average amount of absorption for one hour to be 0.50 gram; from a series of 40 rats given dextrose the average absorption was 0.55 gram (the two series taken together). This is a difference of 9 per cent. In a series of this length, where care has been exercised to overcome all possible sources of error, averages should be indicative of the relative rates of absorption.

In agreement with the findings of Pierce, Osgood and Polansky (5), our results show no relationship between body weight of the rat and absorption. In many instances we observed that a young rat weighing 160 grams would absorb more sugar in one hour than an older rat weighing over 250 grams. The average results of our experiments on rats show a decided decrease in absorption the second hour. The fastest rate of absorption for each sugar is seen in the first thirty-minute period. However, our series is not sufficiently long to make any quantitative statement as to the relative amounts of sugar absorbed during the first and second thirty-minute periods.

CONCLUSIONS

1. Chronic closed loops of ileum in the dog may be used to study absorption. In some respects this method is more dependable than other methods now in use.

2. The three methods used here are in agreement with respect to the relative rates of absorption of dextrose and levulose.

3. Dextrose is absorbed slightly more rapidly than levulose. There is some indication that individual animals may differ in their ability to take up levulose.

4. The rates at which the two sugars are taken up decrease with time. In rats the most rapid absorption takes place during the first thirty-minute period.

5. Our findings show no consistent relationship between body weight of the rat and the amount of absorption over a given time.

BIBLIOGRAPHY

- (1) BURGET, G. E., K. H. MARTZLOFF, R. C. B. THORNTON AND G. R. SUCKOW. 1931. *Arch. Int. Med.*, xlvii, 593.
- (2) CORI, C. F. 1925. *Journ. Biol. Chem.*, lxvi, 691.
- (3) NAGANO, J. 1902. *Pflüger's Arch.*, xc, 389.
- (4) HEWITT, J. A. 1924. *Biochem. Journ.*, xviii, 161.
- (5) PIERCE, H. B., H. S. OSGOOD AND J. B. POLANSKY. 1929. *Journ. Nutrition*, i, 247.

IS LEVULOSE CONVERTED TO DEXTROSE IN THE PROCESS OF ABSORPTION FROM THE INTESTINE?¹

G. E. BURGET, PHILIP MOORE, AND ROBERT LLOYD

From the Department of Physiology, University of Oregon Medical School

Received for publication March 2, 1932

In their studies on hepatectomized animals Bollman and Mann (1931) made the interesting observation that levulose is less effective in preventing the symptoms of hypoglycemia than dextrose. In order that levulose might be effective it was necessary that administration be begun early and larger amounts be given than when dextrose was used. Its efficiency depended upon its conversion to dextrose. It was further found that when the gastro-intestinal tract was removed together with the liver, levulose was without effect in preventing the symptoms of hypoglycemia. The authors made the logical inference that the intestinal tract is capable of bringing about the conversion of fructose to dextrose.

While studying the absorption of sugars from chronic closed loops of ileum in dogs, it occurred to us to further investigate the question of the conversion of fructose to dextrose by the intestinal mucosa. Either by examining specimens from the loop after injecting levulose or by studying the blood from the mesenteric vein from the loop while absorbing the fructose solution definite information should be obtained.

In order to determine amounts of levulose in the presence of glucose the colorimetric method of Corley (1929) was used. This method was found to be quite reliable in determining dilutions up to 0.005 per cent. Where possible, both the polariscopic and Shaffer-Hartman methods were used to further check the findings. When using the Shaffer-Hartman method in the presence of levulose results are somewhat inaccurate. On pure levulose solutions of known concentration this method of determination has been found to give figures under the actual amount by about 10 per cent.

The first observations were made on the aspirated material from loops one hour following the injection of 10 cc. of a 20 per cent solution of levulose. A specimen was used for determining the total reducible sugar by the Shaffer-Hartman method, a cleared specimen was used for a polariscopic reading, and a levulose estimation made by Corley's method. The results of these findings did not indicate the presence of dextrose in the

¹ This work was supported in part by Grant 191 of the Committee on Scientific Research of the American Medical Association.

loop fluid. However, this does not exclude the possibility of the process taking place in the mucosa at the moment of absorption of the molecule. The only way to investigate this possibility was to study the mesenteric blood from the loop during the process of absorption. The dog was given 10 cc. of a 20 per cent solution of levulose via the closed loop. Forty-five minutes later the animal was anesthetized with ether and the abdomen aseptically opened. The mesenteric vein from the loop was exposed and 10 cc. of blood withdrawn. A similar amount of blood was taken from the

TABLE 1

Total reducible sugar and levulose determinations on systemic and mesenteric blood samples one hour after the injection of 10 cc. of a 20 per cent solution of levulose into closed loops in dogs

DOG NUMBER	TOTAL BLOOD SUGAR, SHAFFER-HARTMAN		LEVULOSE IN BLOOD, CORLEY METHOD	
	Mesenteric blood	Systemic blood	Mesenteric blood	Systemic blood
84	0.215	0.203	0.013	None
92	0.214	0.212	++	None
49	0.269	0.237	0.018	None
89	0.213	0.212	+	None
131	0.210	0.185	0.009	None
132	0.210	0.200	0.010	None

TABLE 2

Total blood sugar and levulose determinations on the bath in which a living segment of rabbit's small intestine containing 3M/4 solution has been kept for one hour

LENGTH OF LOOP	AMOUNT OF SUGAR	BATH	TIME	SHAFFER-HARTMAN	LEVULOSE (CORLEY)
cm.	cc. 3M/4	cc.	minutes		
12	3.2	65	60	0.065	0.055
10	3.0	50	60	0.020	0.024
12	4.0	50	60	0.032	0.032
11	3.0	50	60	0.058	0.060
11	3.0	50	60	0.046	0.050
11	3.0	50	60	0.059	0.050

heart. The abdomen was closed and the animal allowed to recover. Total blood sugar and levulose determinations were made on both blood samples. Because of the anesthetic the blood sugar ran high but that is considered of no significance in regard to the levulose content. Table 1 gives the results of experiments on six dogs.

In each experiment the mesenteric blood sugar was greater than that of the systemic blood. Although the method for levulose determination gives fairly accurate results to 0.005 per cent no experiment showed levulose in the systemic circulation. That the sugar was being absorbed is shown by the fact that levulose was present in the mesenteric blood from

the loop. Of course it might be possible that the sugar could be absorbed faster than the conversion took place. The evidence in these experiments, however, would not tend to indicate that such was occurring. The difference between the amount of sugar in the mesenteric and systemic blood samples was in most instances similar to the amount of levulose found in the mesenteric blood.

These results were supplemented by work on isolated segments of small intestine of rabbits. Anchinachie, Macleod and Magee (1930) have shown that living segments of small intestine suspended in isotonic saline solution exhibit the property of selective absorption. Fresh segments were taken from young animals (weight 5 to 6 pounds) and after ligating one end over a rubber covered lead weight and the other over a suitable glass tube 8 to 10 cm. long, the segment was suspended in oxygenated Ringer's solution kept at 37°C. The lead weight was just sufficient to keep the segment immersed in the Ringer's solution. The sugar solution (3M/4) was added via the glass tube. All segments remained active throughout the experimental period of one hour, at which time the bath was examined for total sugar and levulose content. The results are shown in table 2. In four of the six experiments the levulose concentration was found to be as high as the total sugar. If any levulose had been converted to dextrose in passing through the intestinal wall the total reducible sugar would have been greater than the amount of levulose found.

SUMMARY AND CONCLUSIONS

Solutions of levulose were introduced into chronic closed loops of ileum in dogs and into isolated living segments of rabbits' small intestine kept in oxygenated Ringer's solution at 37°C. No glucose was found in the loop fluid after exposure to the mucosa for one hour. The Ringer's solution in which the living segment containing levulose was suspended for one hour showed levulose but no glucose.

Under anesthesia laparotomy was performed upon six dogs and blood taken from the mesenteric vein from the closed loop while absorbing levulose. In all experiments levulose was found in the mesenteric blood while samples of heart's blood taken at the same time showed none.

The evidence obtained from these experiments indicates that levulose is normally taken up by the intestinal tract as such. However, this work does not exclude the possibility that under circumstances such as existed in Bollman and Mann's experiments, the power to convert levulose to dextrose might be called into play.

BIBLIOGRAPHY

- BOLLMAN, J. L. AND F. C. MANN. 1931. *This Journal*, xcvi, 683.
AUCHINACHIE, D. W., J. J. R. MACLEOD AND H. E. MAGEE. 1930. *Journ. Physiol.*,
lxix, 185.
CORLEY, R. C. 1929. *Journ. Biol. Chem.*, lxxxi, 81.

THE AMERICAN JOURNAL OF PHYSIOLOGY

VOL. 101

SEPTEMBER 1, 1932

No. 4

THE PREVENTION OF HYPERTROPHY AND THE LIMITATION OF NORMAL PULSATION AND EXPANSION OF THE KIDNEY BY MEANS OF CASTS¹

SAMUEL SOSKIN AND OTTO SAPHIR

*From the Departments of Physiology and Pathology of the Nelson Morris Institute,
Michael Reese Hospital, Chicago; aided by a grant from the Max Pam Fund and
the John D. Hertz Fund*

Received for publication April 23, 1932

A parenchymatous degeneration (cloudy swelling) of the kidney, liver and myocardium is commonly found at postmortem examination in cases of acute infectious disease. The organs in this condition are much larger than normal, and the parenchyma expands and protrudes when the capsule is incised. The harmful effects of this state of affairs as regards the kidney have been clinically recognized. It is generally accepted that such a kidney, within the intact and relatively inelastic kidney capsule, must have existed under an increased internal pressure, and that this pressure must have limited the blood flow through the organ. Surgical incision of the kidney capsule has been resorted to in an attempt to remedy this condition. It has been customary to assume that the cloudy swelling is responsible for the increased size of the organ and hence for the increased internal pressure, although this belief is entirely based on *post hoc* reasoning. It is quite possible, however, that with the development of an increased pressure within the kidney, with or without the presence of some cloudy swelling, this pressure in itself might not only further incapacitate the kidney but might also be responsible for part of the histological picture observed. In other words, the increased internal pressure may exert a deleterious effect upon the kidney and thus establish a vicious cycle.

In this work we have attempted to study the effects of experimental limitation of increase in kidney volume, and the effects of experimentally produced increased pressure within the normal kidney, without primary kidney damage. The limitation of normal pulsation of the kidney, and of

¹ Presented in preliminary form before the American Physiological Society, April 1931, and the Central Society for Clinical Research, November 1931.

the increase in volume which occurs in this organ with active secretion, was accomplished by surrounding the kidney, in a resting state, with a rigid cast. By removing the opposite kidney, we were able to utilize this well known method of stimulating kidney hypertrophy, to cause an increased pressure within the normal kidney surrounded by the cast. We were thus able to observe the effects of prevention of hypertrophy in a normal kidney. The increased pressure within the cast caused by the attempted hypertrophy must, of course, have produced a further limitation to the normal pulsation and expansion of the kidney.

METHODS. Using an aseptic technic, one or both kidneys of a series of dogs were enclosed in rigid casts applied in the form of narrow gauze bandage soaked in collodium and allowed to harden in situ. In several animals, where the cast was applied to one kidney, the opposite kidney



Fig. 1

Fig. 2

Fig. 1. Cast (opened). Note the space for blood vessels and ureter.

Fig. 2. Kidney bulging through window in cast (other kidney previously removed).

was removed at the same or a subsequent operation, in order to produce an attempt at hypertrophy in the enclosed kidney and hence an increased internal pressure. To determine whether the trauma of the operation or the presence of the foreign material composing the cast were harmful to the kidney, control experiments were performed in which the cast or casts were split as soon as they hardened, but were allowed to remain in situ. Some short-term experiments were undertaken to determine the immediate effects of placing a cast about the kidney upon its urinary output.

In order to establish beyond doubt that the removal of one kidney does cause an increased pressure within the remaining kidney enclosed by a cast, the following procedure was adopted. A cast was applied to a kidney and, as soon as the collodium hardened, a round window, approximately 1.5 cm. in diameter was cut through the cast along its greater

convexity. The opposite kidney was removed at the same operation and the abdomen closed.

In all our experiments, short-term and survival, special care was taken to see that the cast did not interfere with the structures at the kidney hilus. To control the immediate effects of trauma and cooling upon the casted kidney in the short-term experiments, the opposite kidney was always subjected to similar manipulative procedures.

All experiments were concluded by histological examination of the kidneys. Blocks of the kidneys were hardened in 10 per cent formalin and imbedded in paraffin. The sections were stained with hematoxylin-eosin. In all instances, frozen sections from different parts of the kidneys were cut and stained with Sudan III for the presence of fat.

TABLE 1
Survival experiments

SERIES	NUMBER OF DOGS	PROCEDURE	PERIOD OF SURVIVAL
A	3	Cast applied to one kidney; opposite kidney removed at same operation	2-3 days
B	5	Casts applied to both kidneys at same operation	1-22 days
C	5	Cast applied to one kidney; opposite kidney removed 1-2 weeks later	1-14 days
D	1	Cast applied to one kidney. Two months later, cast removed and opposite kidney excised at same operation	5 days
E	13	Cast applied to one kidney. Cast removed at a subsequent operation, 1-5 weeks later. Opposite kidney removed at third operation	5 died (4-30 days). 8 survived "indefinitely"

RESULTS. The results are summarized in the following tables which are self-explanatory. The data presented below are taken from those animals which readily recovered from the operative trauma and which presented no complicating factors, such as infections, etc.

Survival experiments. It will be noted that eight animals in series E, table 1, survived for a length of time which might be termed "indefinite."

The procedure in series D was similar to that in series E except that the opposite kidney was excised at the same instead of a subsequent operation to the removal of the cast. The speedy death of this animal suggested that some interval was necessary for recuperation of the liberated kidney. All subsequent experiments were therefore conducted as in series E.

The procedure applied to dogs of series E, table 1, yielded results (summarized in table 2) which may be divided into three distinct groups:

I. One animal died very quickly with nitrogen retention similar to that shown by the animal with both kidneys enclosed in casts.

II. Four animals died after a relatively short period showing little if any nitrogen retention. It will be noted that in two animals the non-pro-

TABLE 2
*Survival experiments**

Showing detailed observations on dogs in series E, table 1. (Cast applied to one kidney. Cast removed at a subsequent operation 1-5 weeks later. Opposite kidney removed at third operation.)

GROUP	DOG	SURVIVAL PERIOD	BLOOD CHEMISTRY				REMARKS
			Non-protein nitrogen	pH	CO ₂	Total protein	
I {	SSAH†	8 days	140		46.1	23.2	Casts on both kidneys
	SSA1	4 days	130	7.42	34.8	20.5	
II {	SSD	18 days	62	7.65	61.1	13.2	Non-protein nitrogen 256 on 6th day
	SSN	30 days	66	7.43	20.1	16.5	Non-protein nitrogen 92 on 15th day
	SSP	20 days	37	7.70	52.3		
	SSAE	8 days	76	7.40	58.8	15.1	Albumin-globulin ratio = 2.96/1.69
	SSF	Living (429 days)	30	7.57	42.1	17.9	Albumin-globulin ratio = 2.28/3.40
III {	SSAA	Living (230 days)	37	7.50	53.8	16.4	Albumin-globulin ratio = 2.53/5.28
	SSAB	Living (230 days)	31	7.50	48.2	19.3	Albumin-globulin ratio = 3.62/2.79
	SSAD	Living (82 days)	36	7.48	50.6	20.6	
	SSA2	Living (70 days)	71	7.48	43.7	15.7	
	SSA6	Living (82 days)	32	7.43	44.3	17.9	
	SSA7	Living (77 days)	20			17.0	
	SSA8	Living (77 days)	34	7.43	38.0	15.4	

* The chemical data presented represent determinations made within the last 48 hours of life in those animals which died, and within a week from date at which time this table was compiled (January 20, 1932) for those animals still living.

† For purposes of comparison, we include SSAH, an animal with casts applied simultaneously to both kidneys.

tein nitrogen of the blood was higher at an intermediate period than at the time of death. The albumin-globulin ratio of the blood serum was inverted in one dog. The manner of death of these animals will be discussed later.

III. Eight animals survived indefinitely, showing, in general, normal non-protein nitrogen levels and little disturbance in the normal level of other chemical constituents of the blood. It will be noted, however, that dog SSAB shows an inverted albumin-globulin ratio.

Control experiments. The dogs in which the kidneys were surrounded by casts, the casts, however, split as soon as they hardened and allowed to remain in situ, survived indefinitely. This indicates that neither the trauma of the operation nor the presence of foreign material composing the cast is responsible for the results recorded in table 1. Since that portion of the cast which surrounded the kidney hilus was left intact in all of these control animals, it is conclusively shown that our results cannot be due to the encroachment of the cast upon the structures at the hilus. Also, the dog whose one kidney was surrounded by a cast through which a window was cut and whose opposite kidney was removed at the same operation survived for one month, at which time it was sacrificed. At post-mortem examination there was a marked bulging of the kidney parenchyma through the window in the cast, and also a marked eversion of that portion of the cast forming the edges of the window (fig. 2). It is worth mentioning that a similar result was obtained when a cast with a window was applied to a kidney after it had reached a state of considerable hypertrophy following the removal of the opposite kidney. This finding suggests that the attempt at hypertrophy may not be the only change which plays a part in causing the expansion of the kidney through the opening of the cast. The extrusion of kidney substance through the window in the cast and the eversion of the edges of the window show, beyond doubt, that the kidney within the cast must have developed a considerable amount of pressure.

Autopsy findings. In all animals which died with an intact cast enclosing the one remaining kidney, the peritoneal cavity contained a varying amount of a clear or slightly blood stained liquid. Repeated smears from this liquid showed no bacteria. There were marked adhesions between the outer portions of the cast on the kidney and the surrounding structures. Before the casts were removed, the hilus of the kidney was examined in every instance for patency of the arteries, veins and ureters. In no instance was an obstruction of these structures found. The gross and histologic findings in the kidneys were as follows: The organs which had been contained in casts for about two weeks were of normal size and shape, the capsules markedly thickened. The histological examination revealed a varying degree of cloudy swelling and fatty degeneration of the lining cells of the tubuli. In some instances, actual necrosis of these cells was found. These changes were more marked in the tubuli close to the glomeruli. The glomeruli themselves were normal. The kidneys which had been in casts for four weeks or longer were much smaller than normal; they were irregu-

lar in shape due to several bulging areas which were of the same consistency as the neighboring kidney tissue. The histologic examination showed cloudy swelling of the lining cells of the tubuli. Necrosis was much less frequently observed than in the kidneys described before. There was much connective tissue extending from the capsule into the cortex; but no inflammatory changes were found within the glomeruli. The kidneys which had been enclosed in casts for some time, the casts, however, having been removed prior to the death of the animals, showed microscopically a marked fibrosis of the capsule, which extended into the surrounding cortical tissue. There was a moderate infiltration of lymphocytes throughout the cortex. Occasionally, fat droplets were found in the lining cells of the

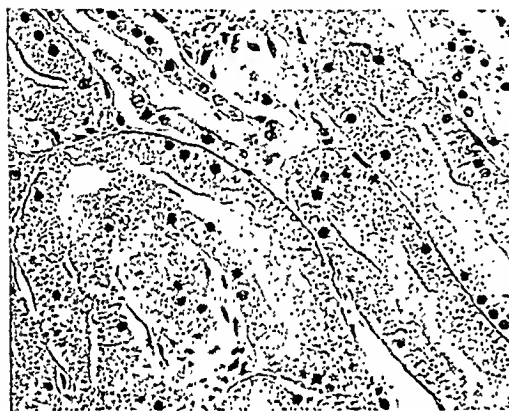


Fig. 3

Fig. 3. Marked cloudy swelling and early necrosis of the tubular epithelium (from a kidney which was left in a cast for a period of two weeks). Hematoxylin-eosin preparation. $\times 260$.



Fig. 4

Fig. 4. Cloudy swelling of the tubular epithelium, and normal glomerulus (from a kidney which remained in a cast for one week). Hematoxylin-eosin preparation. $\times 180$.

tubuli close to the glomeruli. The glomeruli and many tubular lining cells appeared normal. The kidney enclosed in a cast through which a window had been cut showed a distinct bulging of the kidney structure through the window. On section, it was noted that the portion of kidney which projected through the window measured 1.3 cm. in diameter (fig. 2). On histologic examination, there was a slight lymphocytic infiltration throughout the cortex, and some connective tissue proliferation. An occasional glomerulus showed a slight irregularity of its loops, and a few glomeruli contained a reddish granular material in the capsular space. A moderate cloudy swelling of the lining cells of the tubuli was noted. Some of them also contained a few fat globules. Histologic examination of the kidneys

TABLE 3

In this animal, the left kidney was placed in the cast three days before the acute experiment. At the time of the experiment, it may be seen that the encasted kidney was excreting approximately 3 per cent of the amount of urine excreted by the opposite normal kidney. This small amount of urine was very concentrated as to its sugar and nitrogen content. After the cast was removed, the left kidney excreted increasing volumes of urine as compared to the opposite kidney and the urine became proportionately less concentrated. At the end of the experiment, the left kidney was excreting approximately 50 per cent as much urine as the undisturbed kidney.

TIME	MANIPULATION	BLOOD PRES- SURE	NORMAL KIDNEY (RIGHT)						CASTED KIDNEY (LEFT)					
			Volume	Sugar		Nitrogen		Volume	Sugar		Nitrogen		Volume	Total milligrams per cc. urine
				Total milligrams	Milligrams per cc. urine	Total milligrams	Milligrams per cc. urine		Total milligrams	Milligrams per cc. urine	Total milligrams	Milligrams per cc. urine		
		mm. Hg	cc.					cc.						
3-23-31	Cast applied to left kidney													
3-26-31	Abdomen opened and ure-	132												
4:35	ters catheterized under													
	amytal anesthesia													
4:47	50 cc. 10 per cent glucose	144												
	intravenously													
4:49-	Urine collected		47.2	286	6.1	38	0.8	1.5	41	27.3	22	14.7		
5:04														
5:10	Cast removed from left	119												
	kidney													
5:21-	Urine collected		17.65	202	11.5	30	1.7	6.4	157	24.5	25	3.9		
5:36														
5:41	50 cc. 10 per cent glucose	106												
	intravenously													
5:42-	Urine collected	88	10.4	215	20.7	28	2.7	5.1	155	30.4	21	4.1		
5:57														
6:02	0.5 gm. caffeine sodium	91												
	benzoate intravenously													
6:04-	Urine collected		6.3	7	1.1	30	4.8	2.7	6	2.2	24	8.9		
6:19														
6:33	50 cc. 10 per cent glucose	103												
	intravenously. 50 cc.													
	physiological saline in-													
	travenously													
6:35-	Urine collected		8.4	102	12.1	30	3.6	4.0	94	23.5	19	4.8		
6:51														

removed from the control animals in which the casts had been split as soon as applied, but allowed to remain in situ, showed a slight granularity of the cytoplasm of the lining cells of the tubuli in some fields; but many

tubuli appeared normal. No fat could be demonstrated, and there was no evidence of necrosis.

Short-term experiments. In a series of ten dogs, short-term experiments were undertaken to determine the immediate effects of the application and removal of casts on the secretory activity of the kidney. A cast was applied to one kidney of an animal either immediately before or several days before the experiment. The normal kidney was used as a control in each case. Under amytal anesthesia, catheters were inserted into both ureters and the urine excreted by each kidney collected separately and compared as to volume and composition. Table 3 illustrates such an experiment.

DISCUSSION. When a cast was placed on one kidney of a dog and the opposite kidney removed, the animal invariably died. Death resulted whether the nephrectomy was performed at the same or a subsequent operation to the placing of the cast. A similar result was always obtained when both kidneys were simultaneously enclosed in casts. There can therefore be no doubt that the presence of the cast severely impaired the functional activity of the kidney. This is also indicated by the observation that when one kidney had been in a cast for some time, the opposite kidney was found to have hypertrophied. When, however, a cast was applied to one kidney, the cast split as soon as it hardened but allowed to remain in place, and the opposite kidney then removed, the animal survived. Similarly, when casts were applied to both kidneys, and the casts were split as soon as they hardened but allowed to remain in place, the animal survived. Neither the trauma of the operation nor the presence of foreign material around the kidney could therefore have caused significant damage. These control animals also prove that the impairment of kidney function in our experiments was not the result of interference with the patency of the blood vessels or ureter at the kidney hilus.

The survival of the animal whose one remaining kidney was enclosed in a cast through which a window had been cut is very significant. Like the other control experiments, it shows that neither the trauma of the operation, the presence of the foreign material about the kidney nor the interference at the kidney hilus can account for the death of those animals whose kidney or kidneys were surrounded by intact casts. The extrusion of kidney substance through an aperture only 1.5 cm. in diameter, and the eversion of the rigid material around the edges of this opening show, beyond doubt, that the kidney within the cast must have developed a considerable amount of pressure.

When a cast was applied to one kidney, the cast removed two to four weeks later, and the opposite kidney removed at a third operation, 8 out of 13 animals survived. This further indicated that the application of a cast might leave no severe or permanent kidney damage, after the cast

was removed. The death of the animals must therefore be attributed to the volume limitation imposed by the intact cast upon the kidney or kidneys carrying on the renal activity essential to the life of the animal.

The animals which died with their kidney or kidneys enclosed in casts showed a progressively increasing nitrogen retention up to the time of death. Of the animals which were observed following the removal of the cast from their one remaining kidney, only one (group I, table 2) showed such nitrogen retention. The other four animals which did not survive (group II, table 2) showed no significant nitrogen retention. The death of these latter animals, due without doubt to kidney dysfunction, but not associated with nitrogen retention, forms an interesting subject for further study. These animals died in convulsions and in one case where the brain was carefully examined, a marked edema and hyperemia were found. This suggests the clinical diagnosis of so-called "anuremic uremia."

In the short-term experiments, the placing of a cast about one kidney was immediately followed by a marked reduction of its urinary output as compared with that of the opposite free kidney. This occurred even when both kidneys were denervated prior to the experiment, so that nervous inhibition could not have been responsible for the decreased secretory activity of the encased kidney. A markedly reduced urinary output was also observed to occur in kidneys enclosed in casts several days prior to the short-term experiments. However, as soon as the casts were removed from such kidneys, they began to excrete increasing amounts of urine, which soon approached the output of the opposite undisturbed kidneys. The highly concentrated character of the urine excreted by the kidneys in casts or shortly after liberation from such casts is difficult to explain. In view of the fact that the kidney tubules showed the chief histological changes, while the glomeruli were apparently normal, the secretion of a concentrated urine as compared with that secreted by the undisturbed kidney of the opposite side seems difficult to reconcile with the modern reabsorption theory of urinary secretion. Further experiments in this regard are now in progress.

These short-term experiments demonstrate the impairment of kidney function by limitation of expansion, even in the absence of the stimulus to hypertrophy or greatly increased internal pressure. They also show the rapid resumption of function once the cast is removed. It is not difficult to imagine that a similar condition might arise through the restraining influence of the kidney capsule upon the expansion of kidney parenchyma which may be undergoing a sudden cloudy swelling. Once the increased internal pressure is present, the vicious cycle is set in motion leading to more cloudy swelling and a greater increased internal pressure, etc. These acute experiments suggest a simple mechanical explanation for the occurrence of some cases of temporary oliguria or anuria. Since this explanation

does not depend upon the assumption of a severe pathological process in the kidney, it seems especially applicable to those instances in which the kidney dysfunction disappears as rapidly as it appeared, leaving no evidences of marked kidney damage. This might be accounted for by the breaking of the vicious cycle, either through the stretching of the kidney capsule with time, or the alleviation of the original cause of the initial cloudy swelling.

SUMMARY

In a series of dogs, kidneys were aseptically enclosed in gauze and colloidum casts, care being taken to avoid interference with the blood vessels and ureter at the hilus. When one kidney of an animal was so treated and the other kidney removed, at the same or a subsequent operation, the animal invariably died. If, however, the cast was removed from the one kidney before the other kidney was extirpated, the animal often survived. Various control experiments indicated that the procedure employed caused an increased internal pressure within the kidney enclosed by the cast, and that neither the trauma of the operation nor the presence of the foreign material about the kidney appreciably influenced the results obtained.

These experiments show that a significant impairment of functional activity occurs in kidneys surrounded by a rigid cast. This impairment does not depend on extensive or permanent kidney damage. These results therefore indicate that the procedure which we have employed interfered with normal physiologic conditions necessary to kidney function. Such conditions may be:

1. The normal pulsation coincident with the pulse pressure
2. The normal expansion during active secretion
3. The hypertrophy stimulated by removing the opposite kidney or incapacitating it with a cast.

Although the prevention of hypertrophy may have played a rôle in the survival experiments, the short-term experiments indicate that the interference with normal pulsation and expansion of the kidney was probably the chief factor involved. The increased internal pressure resulting from the attempt at hypertrophy within the rigid cast must have increased the limitations imposed by the cast. It is not improbable that similar conditions are responsible for some of the functional impairment and pathological changes in kidneys which are ordinarily considered to be manifesting the results of a simple parenchymatous degeneration. The appearance of this cloudy swelling in normal kidneys, as a result of the conditions set up by our procedure, is significant.

THE PHYSIOLOGICAL ACTIVITY OF IODINE IN THYRO- GLOBULIN

BRODA OTTO BARNES

From the Department of Physiology, University of Chicago

Received for publication May 18, 1932

Attempts to prepare a characteristic substance from the thyroid gland began certainly as early as 1884 when Bubnow (1) prepared "Thyreoprotein." Langerdorff (2) made some observations on the colloid of the thyroid and noticed that it became readily soluble when subjected to artificial gastric digestion. Gourley (3) prepared a substance which he called the "principal proteid" of the thyroid. From the properties of his compound, it must have been the same as Bubnow's preparation. It remained for Baumann (4) to discover iodine in the gland in 1895. His correlation of the iodine content with diseases of the thyroid not only paved the way for iodine therapy but also gave a chemical means of following the active principle in thyroid preparations. Attempts to concentrate or isolate iodine-containing compounds characteristic of thyroid action may be classified under the three following heads: acid hydrolysis, alkaline hydrolysis, and by the use of enzymes. Only the latter will be considered here since it is closer related to experimental hyperthyroidism produced by feeding thyroid preparations.

Most of the work involving enzyme hydrolysis is open to one of two criticisms: either very little was done in attempting to separate the iodine compound or else the fractions were not properly assayed. Hutchison (5) as early as 1896 reported that the protein which Bubnow and Gourley had described contained iodine. He carried out a series of experiments on this compound including the solubility, color reactions, heat of coagulation, hydrolysis with acid, and the effect of gastric digestion. By the latter method, he separated the iodine into two fractions which were then assayed on thyroidectomized dogs in tetany. His conclusions are unwarranted in view of the present conception of tetany and the parathyroids. The same author (6) later reported the separation of digested thyroid into three fractions: iodothyron, albumoses and peptones. The iodine content decreased in the order mentioned. They were tested on a case of myxedema and their effect on loss of weight was proportional to their iodine content. Tamback (7) studied the effect of both pepsin and trypsin on

thyroid preparations, but did not study their physiological activity. Some digestion studies on human thyroids were carried out by von Cyon and Oswald (8), but their products were tested on the circulation and not on thyroid deficient animals. Oswald (9) in 1908 reported quantitative studies on thyroglobulin digested with trypsin. His report that as much as two-thirds of the iodine might be liberated in inorganic form has probably kept many workers from using enzymes. However, it must be remembered that the active principle is not destroyed, at least entirely, when thyroid is fed. Furthermore, Oswald did not examine his mixtures until months after their preparation. The length of time and possible bacterial contamination might have added to the decomposition. Nurnberg (10) carried out some extensive chemical studies on the products after digestion of thyroglobulin, but he did not test them physiologically. Pick and Pineles (11) were the first to combine both separation and adequate assay. Using thyroidectomized goats which displayed cretin symptoms, they found that fresh thyroids, thyroglobulin, and secondary albumoses relieved the thyroid deficiency. On the other hand, iodothyryn, primary albumoses, and digestive mixtures standing three months were ineffective. Cameron and Carmichael (12) made the first attempts to compare the effects of undigested thyroglobulin with the products of digestion. Although they state that their method of assay, using body weight and hypertrophy of the kidneys, liver and heart, is not quantitative, the digested mixture had the same order of activity as the original protein. They examined the residue after digestion, the filtrate, and, in one case, the peptones prepared from the filtrate. All of these fractions were active by their assay. Last year Harington and Salter (13) employed enzymes for the isolation of thyroxine from thyroglobulin. They discarded the filtrate at pH 5.0, apparently without testing its potency. This is not surprising, since Kendall (14) had reported that the acid-soluble fraction after alkaline hydrolysis was inactive. Harington and Randall (15) had confirmed this and shown that thyroxine was quantitatively precipitated at pH 5.0.

Numerous reports have been made that, in equivalent amounts of iodine, thyroxine is less active than desiccated glands. On the other hand, it seems definitely agreed that only a portion of the iodine in the thyroid is present as thyroxine. Therefore one must postulate either that the active principle is more potent than thyroxine per se or that other compounds are present in desiccated thyroid which add to the effects of thyroxine. A study was undertaken to test various fractions of artificially digested thyroglobulin. Since it was found that the filtrate at pH 5.0, after tryptic digestion, might contain as much as fifty per cent or more of the total iodine, this filtrate was examined in some detail for its physiological actions.

METHODS. 1. *Preparation of thyroglobulin digest.*¹ The thyroglobulin used was prepared by a method to be described later. The principle involved the precipitation of the protein from thyroid extract at the isoelectric point. A portion of the thyroid extract was reserved in order to compare the effects of the original active principle with it after digestion. The precipitated thyroglobulin was put into one per cent sodium bicarbonate, and commercial pancreatin was added to a concentration of 0.2 per cent. A few drops of alcoholic thymol were added as a preservative. Although the bicarbonate tends to keep the reaction of such a mixture slightly alkaline, the pH was adjusted daily to 8.0. The pancreatin was renewed after 48 hours. After being in the incubator for 72 hours, the mixture was brought to a pH of 5.0, and the precipitate was collected on a filter. This procedure differs from Harington's only in the preparation of thyroglobulin and in the presence of the bicarbonate. A control solution was prepared by digesting casein in a similar manner. However, this digest was not fractionated.

2. *Work on rats.* Since the digested filtrate might contain toxic substances, it was administered orally by a stomach tube. This was accomplished under light ether anesthesia. The animals were placed on their backs and a small tube attached to a syringe was gently pushed down through the esophagus into the stomach. The fluid could then be injected without any trouble or danger of loss. The dosage varied from 2 to 4 cc. depending on the size of the animal. The procedure was so simple that the rat was under the anesthetic only 2 or 3 minutes.

3. *Work on rabbits.* Two rabbits were given the filtrate by means of a stomach tube, and two others were given the dried precipitate put into capsules. No anesthesia was necessary. When the capsules were given, the animals were observed closely until they had swallowed all of the material. The body weight and rectal temperature were recorded daily.

4. *Work on dogs.* Three dogs were employed for basal metabolism studies. They were trained to lie quietly, and the calories per 24 hours were determined using the Benedict portable apparatus. After control observations had been made (at least 10), the filtrate was given to two of the dogs by stomach tube. Five doses were administered and the heat production, heart rate, respiration and rectal temperature determined. At the end of this experiment, one of these dogs and the third one were fed the precipitate after digestion of the thyroglobulin. The quantities of both the precipitate and the filtrate employed contained the same amount of iodine.

RESULTS. In a preliminary experiment, two preparations of the filtrate at pH 5.0 were prepared and tested for thyroid activity by the loss of

¹ I am indebted to Armour & Company for the fresh thyroids.

weight in rats. Four animals were given each preparation, and four animals were anesthetized in the same way and given a similar quantity of tap water as a control. Of the 8 experimental animals, all lost weight at the beginning but recovered somewhat at the end of 3 weeks. The weight losses amounted to as much as 20 grams in several cases, and one animal died with a loss of 28 grams. At the end of three weeks only one animal was heavier than at the beginning, while the controls had gained weight consistently. These experiments indicated that there was some thyroid-like activity in the filtrate. It was then decided to use more rats, rabbits, and basal metabolism tests on dogs in order to investigate this indication thoroughly.

Experiments on rats. To examine further the effects of ether on growth and the body weight, forty white rats ranging from 70 to 130 grams in weight were divided into two groups. Half of these were anesthetized daily for a period of 7 days. Neither group showed any weight loss during the week, and at the end of the experiment the controls had gained an average of 15.7 grams while those anesthetized averaged 13 grams. If ether did retard growth, certainly it was to a minor degree. More animals would have to be employed to be sure that there was any significant effect. A few days intervened before the same animals were divided into three groups, two of ten each and one of twenty. One group of ten received the original thyroid extract, the other small group received the same volume of casein digest. The large group was given the same volume of digested thyroglobulin filtrate, which contained the same quantity of iodine as the original thyroid extract. The dosage was regulated so that it contained an amount of iodine equivalent to two grams of U. S. P. desiccated thyroid per kilo body weight. The average daily variation for each group is recorded in figure 1. It can be seen that all three groups lost weight at the beginning, but the effect was much greater with the thyroid extract. No explanation can be given for the loss of weight in the animals on casein, where growth was retarded, since at the end of the week they had gained only 4.7 grams. According to the experiment where only ether was given, the gain should have been 13 grams. Since this weight loss has been obtained once with casein and three times with thyroglobulin fractions which contain very little if any thyroxine, one is forced to raise the question whether toxic substances which cause loss of weight are produced by artificial digestion. No attempt will be made to answer this question at present, but the loss of weight in rats has been abandoned as a method of thyroid assay.

The group of animals on the filtrate from digested thyroglobulin lost a little more weight than those on casein, and at the end of the week were only back to their original weight. One cannot interpret this as an indication of thyroid activity in view of what has just been said. However,

their weight loss was in no way comparable with that of the animals on thyroid extract. Here the weight loss was much more severe and five of the animals had died within four days. On the seventh day the dosage of both the original extract and the digested filtrate was increased. Three days later only two of the ten rats on the extract were alive and only one from the group of 20 had died. This latter animal had lost no weight and probably died from other causes. These results clearly show that the digested filtrate contains very little thyroid activity in comparison to the original extract, when equal quantities of iodine are administered.

Experiments on rabbits. The dosage in terms of iodine for the rabbits receiving the filtrate was equivalent to 2 grams of desiccated thyroid per kilo. Unfortunately one of these animals was killed accidentally early

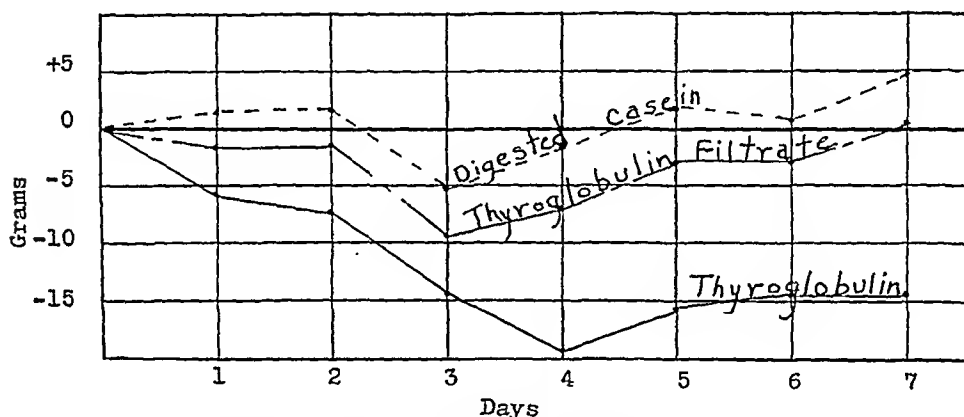


Fig. 1. Showing the average daily changes in weight of rats fed digested casein, undigested thyroglobulin, and the filtrate obtained by bringing digested thyroglobulin to pH 5.0.

in the experiment. The data are summarized in table 1. The rabbit receiving the filtrate continually lost weight during the ten-day period. Although in this case a total of over 400 grams was lost, there was never any diarrhea observed. After the experiment stopped the animal started to regain weight, but observations were not continued until recovery was complete. This experiment furnishes further evidence that there is a factor causing loss of weight, and in this instance there was no anesthesia. There seems a significant difference, however, between the rats and the rabbits. The rats make some adjustment to the toxic factor while the rabbits do not, at least within ten days.

The two rabbits fed the precipitate from thyroglobulin digest exhibited quite a different reaction. Their weight loss was so severe that only three doses were given. The dosage in this case was only three-fourths as great, or the equivalent of one and one-half grams of desiccated thyroid

per kilo. Both animals had severe diarrhea from the second day until they died. When their weights had dropped over 400 and 300 grams respectively in 3 days, the feeding was stopped in order to allow recovery. However, the weather was hot at this time and the animals did not survive the toxicity, one dying the following day and the other 24 hours later. This rapid loss of weight would lead one to believe that the precipitate was very active physiologically, but no quantitative comparisons can be made.

No change in the temperature of the animal receiving the filtrate was ever noticed. Daily fluctuations with changes in the weather were the same as in the control animals. The same may be said for animal 4. The day before animal 3 died his temperature was 2°C. higher than at any other time. In summarizing the data on rabbits, it would appear

TABLE 1

The daily weight of rabbits fed the filtrate and the precipitate obtained by bringing digested thyroglobulin to pH 5.0

DAYS	FILTRATE		PRECIPITATE	
	No. 1	No. 2	No. 3	No. 4
0	2,420	1,740	1,960	1,960
1	2,360	1,700	1,900	1,930
2	2,320	1,620	1,680	1,740
3	2,240	Killed	1,540	1,620
4	2,200		Dead	1,560
5	2,140			Dead
6	2,160			
7	2,120			
8	2,060			
9	2,000			
10	1,960			

that there is some factor in the filtrate which causes loss of weight but does not cause diarrhea or elevation of body temperature. The precipitate causes a much more rapid loss of weight, severe diarrhea, and may elevate body temperature.

Experiments on basal metabolism. Both the filtrate and precipitate were tested for their effect on basal metabolism, since it is probably the final test for thyroid activity. Large doses which were equivalent in iodine to 13 grams of desiccated thyroid were employed. Five daily doses were given to dogs 1 and 2. It can be seen in table 2 that the filtrate had no effect on basal metabolism. This was a surprise since the weight loss in both rats and rabbits had indicated some activity. Just how little hormone is necessary to raise basal metabolism cannot be stated, but from the work of Kunde (16) it would appear that there was not much, if any,

of the active principle present. There was no change in heart rate, temperature, or respiration that would indicate any hyperthyroidism.

When the precipitate having an amount of iodine equivalent to that in the filtrate was fed the basal metabolisms showed a significant rise within 24 hours. Dog 2 showed a gradual rise until on the third day the heat production was over 31 per cent above the controls. Only three doses were fed after which the basal metabolic rate came down gradually. On the seventh day, or 4 days after the last feeding of the thyroid preparation, the basal metabolism was within the control range. Dog 3 did not reach the high peak until the fourth day although none of the material

TABLE 2

The daily changes in basal metabolism and heart rate produced by feeding the filtrate and precipitate obtained by bringing digested thyroglobulin to pH 5.0

DAYS	DOG 1		DOG 2		DOG 3		REMARKS
	Calories per 24 hours	Heart rate	Calories per 24 hours	Heart rate	Calories per 24 hours	Heart rate	
0	341.3	102	330.5	61	439.0	54	Average of controls
1	335.1	105	334.2	62			Given 100 cc. filtrate
2			325.6	60			Given 100 cc. filtrate
3	344.7	93	329.6	60			Given 100 cc. filtrate
4	340.6	80	326.6	62			Given 100 cc. filtrate
5	322.3	78	353.2	60			
0							1.1 gm. pt. fed
1			366.8	70	483.7	74	1.1 gm. pt. fed
2			387.9	84	479.9	64	1.1 gm. pt. fed
3			433.7	84	512.6	73	Fed no thyroid
4			411.3	74	600.5	79	Fed no thyroid
5			389.9	68	504.0	63	Fed no thyroid
6			356.2	62	492.0	58	Fed no thyroid
7			347.2	58			

was fed on the previous date. The return to normal was also slower than the other dog. The heart rate paralleled the basal metabolism in both cases and was surprisingly close in dog 2, whose normal rate was quite constant and never varied over 4 beats per minute in the control period. The heart rate in each case returned to normal before the heat production. In dog 2 the temperature was elevated 0.5°C. on the third, fourth, and fifth days. Dog 3 showed a rise of 0.2 degree only on the fourth day, when his basal metabolism was also at its peak. Respiration did not parallel the heat production as closely as the heart beats, but the respiratory rate on the day of maximum basal metabolism was double the rate at any other time.

Although these observations indicate that the precipitate has considerable potency, no quantitative comparisons can be made. It is apparent that the iodine of the thyroglobulin can be split into two definite fractions by the use of pancreatin. Since one of these fractions shows comparatively little, if any, activity, it would seem conclusive proof that all of the iodine is not associated with the active principle. This agrees with the evidence of Kendall and Harington that all of the iodine is not present as thyroxine. However, the evidence that desiccated thyroid is more active per iodine content than thyroxine has not been satisfactorily explained. Further studies on the precipitate will be made in order to see if some of the active principle might have been destroyed by the artificial digestion.

SUMMARY

1. By the use of pancreatin, thyroglobulin was split into an acid-soluble fraction and an acid-insoluble fraction. The iodine content of each fraction was about the same in some cases. The acid-soluble fraction caused a loss of weight in rats and rabbits. It does not cause elevation of body temperature, increase in basal metabolism or diarrhea.

2. The acid-insoluble fraction caused much greater weight losses, severe diarrhea, elevation of body temperature, increased heart rate, rapid respiration, and a rise in basal metabolism.

3. If artificial digestion has not destroyed any of the physiological activity, at least only half of the total iodine in the thyroid is combined with the active principle.

I wish to take this opportunity to thank Mr. G. F. Stewart for making the pH adjustments for me. It has been a great opportunity and a pleasure to carry out this work under the direction of Dr. A. J. Carlson, whose advice and encouragement have meant so much.

BIBLIOGRAPHY

- (1) BUBNOW. *Zeitschr. f. physiol. Chem.*, 1884, viii, 1.
- (2) LANGENDORFF. Quoted from HUTCHISON.
- (3) GOURLAY. *Journ. Physiol.*, 1894, xvi, 23.
- (4) BAUMANN. *Zeitschr. f. physiol. Chemie*, 1895, xxi, 319.
- (5) HUTCHISON. *Journ. Physiol.*, 1896, xx, 474.
- (6) HUTCHISON. *Journ. Physiol.*, 1898-99, xxiii, 178.
- (7) TAMBACK. *Zeitschr. Biol.*, 1898, xxxvi, 549.
- (8) VON CYON AND OSWALD. *Pflüger's Arch.*, 1901, lxxxiii, 199.
- (9) OSWALD. *Arch. f. exper. Path. u. Pharm.*, 1908, lx, 115.
- (10) NÜRNBERG. *Biochem. Zeitschr.*, 1909, xvi, 87.
- (11) PICK AND PINELES. *Zeitschr. exper. Path.*, 1909-10, vii, 518.
- (12) CAMERON AND CARMICHAEL. *Trans. Roy. Soc. Canada*, 1926, xx, 307.
- (13) HARINGTON AND SALTER. *Biochem. Journ.*, 1930, xxiv, 456.
- (14) KENDALL. *Journ. Biol. Chem.*, 1915, xx, 501.
- (15) HARINGTON AND RANDALL. *Biochem. Journ.*, 1929, xxiii, 373.
- (16) KUNDE. *This Journal*, 1927, lxxxii, 195.

MANGANESE AS A FACTOR IN REPRODUCTION¹

J. T. SKINNER, EVELYN VAN DONK² AND H. STEENBOCK

From the Department of Agricultural Chemistry, University of Wisconsin, Madison

Received for publication May 25, 1932

Because of its low content of certain inorganic elements, notably iron, copper and manganese, whole milk has been used extensively as a basal ration in studies of the functions of these elements in animal nutrition. Painstaking attempts to produce basal rations lower in these elements by using purified food materials end frequently in synthetic rations in which these elements are present in greater concentrations than in cow's milk.

For various reasons, some of which have been satisfactorily explained, milk does not support normal reproduction. In 1925 Daniels and Hutton (1) reported that reproduction in milk fed animals was benefited by the addition of small amounts of certain inorganic elements. It is now known that iron and copper must be added to a milk diet in order to maintain the hemoglobin level which, obviously, is necessary if normal reproduction is to be secured. Waddell, Steenbock and Hart (2) found that reproduction was far below normal on a milk diet supplemented with iron and copper, and Krauss (3) secured no young from rats reared on such a diet. Keil and Nelson (4), obtained young from females receiving milk supplemented with copper and iron, but observed that lactation was frequently inadequate to permit the mothers to rear their offspring.

One phase of reproduction which lends itself readily to accurate observations over even relatively short periods of time is that of oestrus. Observations by Waddell et al. (2) on the oestrous cycle in the rat when receiving iron and copper as supplements to whole milk, indicated that oestrous cycles, if they occur at all, do so at rather long and irregular intervals. Kemmerer, Elvehjem and Hart (5) noted similar performances by female mice on such a ration. In both of these investigations it was observed that manganese, when added to the milk-iron-copper diet increased the frequency of oestrus. The conclusions regarding the effect of manganese upon the oestrous rhythm in the rat (2), unfortunately, were based upon preliminary observations in which an adequate number of animals was not available, and no controls were run simultaneously. The work de-

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

² E. R. Squibb and Sons Fellow.

scribed herein is a continuation of this phase of the study of reproduction, in which sufficient animals have been used to warrant more positive conclusions.

EXPERIMENTAL. For these experiments 37 young female rats were selected from litters reared by mothers which were put on an exclusive milk diet when the young were 12 days of age. This precaution was observed in order that the animals would have the least possible stores of manganese at the beginning of the experimental period. Twenty-eight of them were placed in the control group, which received milk *ad libitum* plus 1.0 mgm. of iron and 0.5 mgm. of copper per 100 cc. The other 9 received in addition to the diet of the controls, manganese to the extent of 1.0 mgm. per 100 cc. of milk. The iron was fed as ferric chloride and was prepared from standardization iron wire according to the usual procedure (6). The C. P. grades of the sulfates of copper and manganese were employed.

One of the criteria used in determining the effect of Mn was that of incidence of sexual maturity. Mention has been made elsewhere (7) of the relative rates of growth of the two groups of animals during the first 10 weeks on the experimental rations. Since sexual maturity is usually retarded in animals which do not grow normally, it was expected that in animals receiving the manganese supplement the vaginal orifice would be established at an earlier age than in the controls. Of the 28 females in the control group 5 were changed to a second supplement before this stage of development had been reached; therefore the comparison using this as a criterion must be limited to the performance of the remaining 23.

Examination of the growth curves in charts I and II shows that there were outstanding exceptions to the relationship which usually exists between the rate of growth and attainment of sexual maturity. For the 23 females maintained on the milk-iron-copper diet the average age attained before the vagina opened was 100 days; the limits were 60 and 188 days. The average would have been greater had the other five been continued on the control diet, for they were 128 days old when changed to the supplemented diet. The age of the manganese animals ranged from 61 to 131 days and averaged 87 days when the vaginal orifice was established. Opening of the vaginal orifice prior to the hundredth day was noted in only 39.2 per cent of the controls as compared with 66.6 per cent of those receiving manganese.

In chart I are superimposed on the growth curves a record of the oestrous cycles of 12 animals which were maintained on the control diet, and 9 which received an additional supplement of manganese. In order to conserve space and since our primary interest is in presenting the frequency of oestrus, the growth curves during the first 60 days of life have not been included.

A study of chart I reveals that, on the whole, oestrus was poor when iron

and copper comprised the only supplement for milk. Animal 132 was an outstanding exception from the beginning, she having matured early and exhibited oestrus at regular intervals over a period of 4 months. In addition to this female, 6 others also showed fairly regular oestrus during the

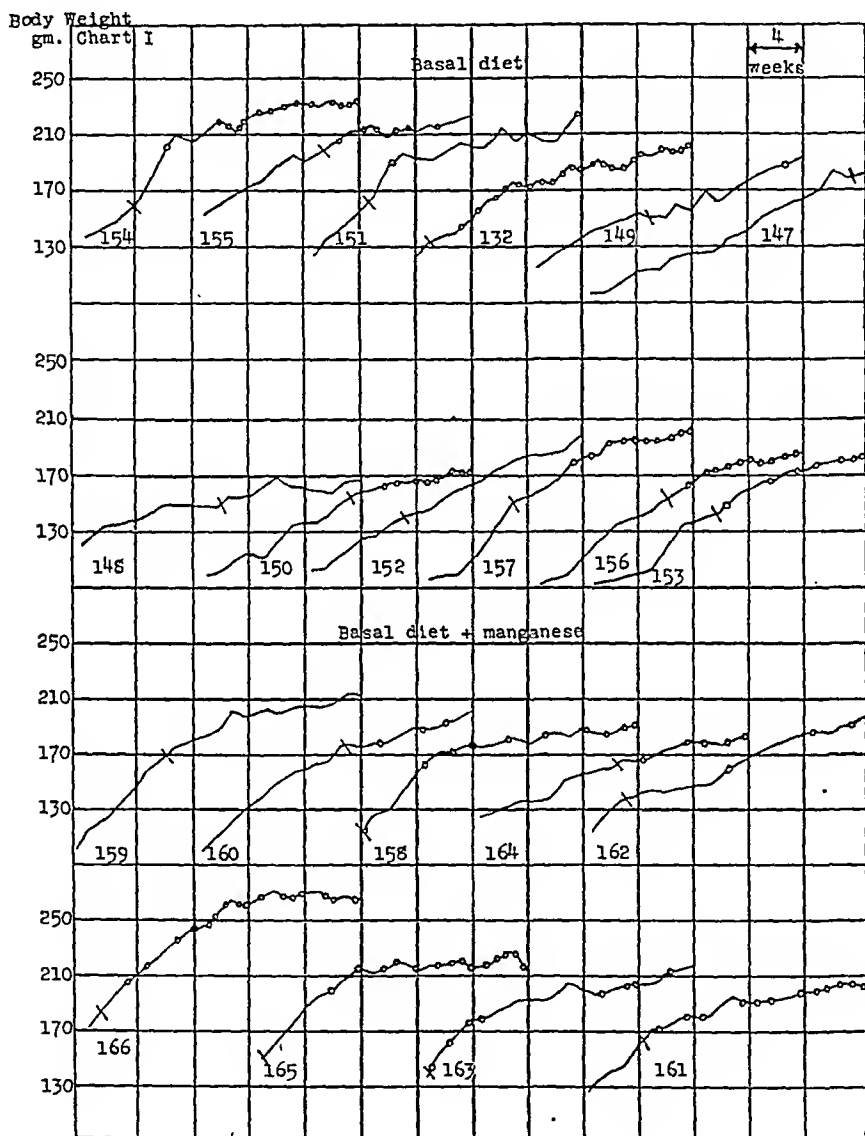


Chart I. Effect of manganese on oestrus. Basal diet: milk *ad libitum*; iron, 1.0; and copper 0.5 mgm. per 100 cc. of milk.

Supplement: manganese 1.0 mgm. per 100 cc. of milk.

In this and the following chart the cross lines on the curves indicate establishment of the vaginal orifices.

It is evident that manganese was ineffective in producing normal oestrus.

last 2 months of the experiment, but the average cycle for this period was decidedly longer than normal, viz., 7.5 days. It is improbable that these

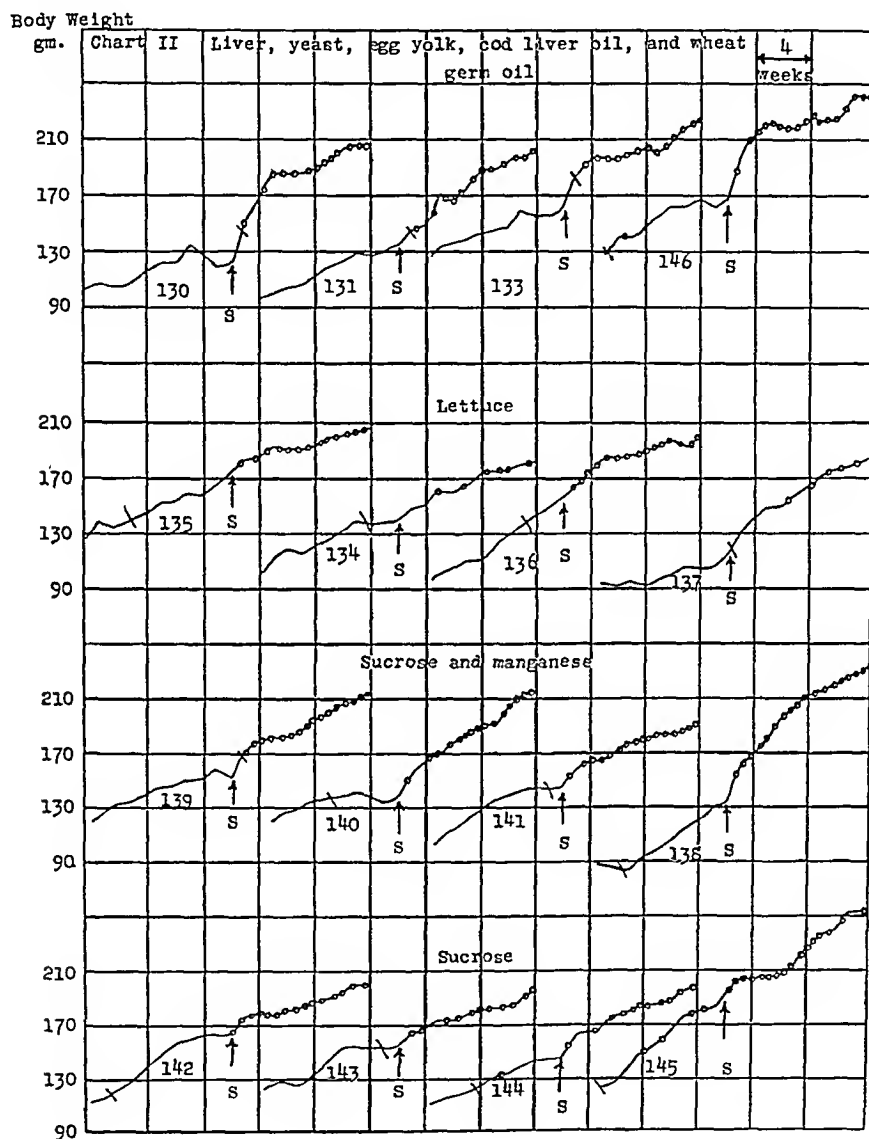


Chart II. Basal diet: milk *ad libitum*; iron, 1.0; and copper 0.5 mgm. per 100 cc. of milk.

Supplements at points *S*: 1. food mixture consisting of liver, 3; yeast, 2; egg yolk, 2; cod liver oil, 0.5; and wheat germ oil, 0.5 gram per 100 cc. of milk. 2, Five grams of lettuce per animal daily. 3, Ten grams of sucrose and 1.0 mgm. of manganese per 100 cc. of milk. 4, Ten grams of sucrose per 100 cc. of milk.

The sucrose-manganese supplement was most effective in promoting normal oestrus. Each of the other supplements, although to a lesser extent, also tended to promote a normal oestrous rhythm.

females obtained additional substance or substances by way of contamination since they were caged with other animals in which oestrus never occurred. The difference in behavior must therefore be attributed to variations in individuals. Further evidence that animals on a milk-iron-copper diet do not show the normal oestrous cycle was obtained on the other 16 animals which, prior to the last 10 weeks, were kept on this diet. Mention will be made of them later in connection with a discussion of chart II.

As is evident in the lower half of chart I, where the curves for the manganese supplemented group are given, the effect of this element was not what would have been expected from the work cited above (2). Indeed there was little difference between the oestrous rhythm in this group and the control group. Although a greater percentage of these animals exhibited oestrus three or more times, there were some control animals which performed as well as any which received manganese.

Effect of various food materials on oestrus. When it became apparent that additions of manganese to the milk-iron-copper diet were ineffective in regularly promoting normal oestrus, it was decided to try substances other than single mineral elements. Consequently, 16 of the animals which had been reared in the control group and which had shown no indication of normal oestrus were divided into 4 groups and the control diet was supplemented as follows: 1, a food mixture consisting of liver, yeast, egg yolk, cod liver oil, and wheat germ oil; 2, head lettuce; 3, sucrose and manganese; and 4, sucrose alone. In the selection of these supplements we were guided by previous work in this laboratory (8) which had indicated that oestrus was more regular in animals on a milk-iron-copper diet when the caloric intake was increased. An effort was made to supply the first group with additional energy, potent sources of the known vitamins, and an animal food rich in minerals. Enough of the supplemental mixture was prepared at a time to last 3 or 4 weeks and stored at a temperature below freezing, the daily requirement being removed as needed. The supplement of the second group, viz., head lettuce, should have furnished any requisite supplementary minerals as well as small amounts of some of the vitamins. Unlike the first two supplements which might have contained one or more specific substances requisite for normal oestrus, the other two supplements were designed primarily to increase the energy intake. The possibility that a pronounced response to such treatment might depend upon the presence of more manganese in the diet than the milk would provide led us to include this also in one of the supplements. The performances of these 4 groups are shown in chart II.

It is evident from a study of the upper half of the chart that frequency of oestrus was increased when either the food mixture or lettuce was added to the basal diet. During the 10 week period that the 4 animals were on the former oestrus occurred every 5 days, due allowance being

made for the time elapsing previous to establishment of the vaginal orifice. Previously the one sexually mature animal had exhibited oestrus only once over a period of 2 months and the remaining three, although 128 days of age when the supplement was added, were yet sexually immature. It will be noted that each of these reached maturity within a week after the addition of the supplement. Lettuce proved less effective than the food mixture. Whereas in two of the animals oestrus began to occur regularly soon after the addition of lettuce, the performance of the other two was subnormal throughout the entire period. However, it should be noted that oestrus had not occurred in either of the 3 sexually mature animals while they were on the exclusive milk-iron-copper diet.

The performances of the animals in both groups receiving sucrose, which are recorded in the lower half of chart II, indicate that for normal oestrus the prime deficiency of the milk-iron-copper-ration is that of the energy. Whereas in the sucrose-manganese group oestrus had not been observed in any animal prior to the addition of this supplement, the average rate of appearance of oestrus thereafter was once every 4.3 days. Animals receiving sucrose but no manganese exhibited somewhat longer, though regular, cycles of 5.4 days' duration. It should be mentioned that sucrose was found to contain only 0.021 mgm. of manganese per kilo. Actually the total manganese intake of the animals which received the sucrose supplement was less than that of the controls due to a compensatory decrease in the consumption of milk by the former. It is therefore evident that energy was the prime determinant. However, the maximum supplementary effect of sucrose was not elicited without manganese additions.

Records which were obtained during the last 26 days of feeding show that the approximate intake of energy per animal in each group during this period was: control, 830; manganese, 840; food mixture, 880; lettuce, 930; sucrose-manganese, 1130; and sucrose, 1110 Calories.

It is unfortunate that no records were kept of the food consumption of the various groups for the periods immediately following the change of supplements. Greater differences in the amounts of food ingested may have occurred at that critical stage than later in the experiment. The data, as far as they go, show that the animals on the sucrose and sucrose-manganese supplements, in which oestrus was regular, consumed approximately $\frac{1}{3}$ more calories than the controls. On the other hand, this relationship does not hold for the other groups in which the frequency of oestrus was increased, and our present information permits no conclusion for these groups as to the factor or factors responsible for the improvement.

SUMMARY

Female rats reared on whole milk fortified with copper and iron did not attain sexual maturity, as indicated by establishment of the vaginal orifice, as early as those receiving the same ration supplemented with manganese.

Females receiving a manganese supplement did not exhibit normal oestrous cycles. Like those on the milk-iron-copper diet they often failed to exhibit oestrus over long periods of time and when cycles occurred, they were less frequent than in the normal animal.

Females receiving sucrose and manganese in addition to the basal ration exhibited normal oestrous cycles of 4.3 days' duration. Those given sucrose but no manganese performed less satisfactorily. When a food mixture (liver, yeast, egg yolk, cod liver oil and wheat germ oil) was added to the basal diet, oestrous cycles occurred at intervals slightly longer than normal, namely, 5 days.

BIBLIOGRAPHY

- (1) DANIELS, A. L. AND M. K. HUTTON. *Journ. Biol. Chem.*, 1925, lxxiii, 143.
- (2) WADDELL, J., H. STEENBOCK AND E. B. HART. *Journ. Nutri.*, 1931, iv, 53.
- (3) KRAUSS, W. E. *Journ. Dairy Sci.*, 1929, xii, 242.
- (4) KEIL, H. L. AND V. E. NELSON. *Journ. Biol. Chem.*, 1931, xciii, 49.
- (5) KEMMERER, A. R., C. A. ELVEHJEM AND E. B. HART. *Journ. Biol. Chem.*, 1931, xcii, 623.
- (6) WADDELL, J., H. STEENBOCK AND E. B. HART. *Journ. Biol. Chem.*, 1929, lxxiii, 243.
- (7) SKINNER, J. T., W. H. PETERSON AND H. STEENBOCK. *In press.*
- (8) VAN DONK, E. AND H. STEENBOCK. *In press.*

I. THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON GASTRO-INTESTINAL MOTILITY¹

DOROTHY FETTER AND A. J. CARLSON

From the Physiological Laboratory of the University of Chicago

Received for publication May 26, 1932

Hyperthyroidism as it is observed in the clinic is frequently accompanied by gastro-intestinal symptoms, the most common of which are increased desire for food, and diarrhea. Since in hyperthyroidism oxidation is increased, more food is necessary than normal in order that the tissues of the body should not be used. A hyperthyroid patient may require a diet containing as many as 5,000 calories in order to maintain his body weight (1). Exercise of any kind is accomplished with a greater expenditure of calories than normal. Therefore it may be assumed that the increased desire for food is an adjustment to a new bodily demand, and is a generalized sensation. Carlson has shown that hunger is a sensation accompanying movements of the empty stomach (2); in conditions where hunger is abnormally keen, contractions of the stomach are increased in height and frequency (3). Presumably then the increased desire for food in hyperthyroid patients may be related to an increased motor activity of the empty stomach. Perussé and Rozen (4) have studied the activity of the empty stomach of dogs during periods of feeding with desiccated thyroid, and found that the motility varied, depending upon the amount of thyroid administered. Kratinoff (5) has also studied the effects of induced hyperthyroidism upon the activity of the stomach and duodenum of dogs, and found in some cases an elevation, and in some cases a depression of the motility.

The diarrhea of hyperthyroidism also indicates an increased motility of the digestive tract. King (6) mentions diarrhea as one of the most frequent and annoying symptoms of the disease. He attributes it to the lack of free hydrochloric acid which allows the pylorus to stay open and food to pass on before digestion has occurred, thereby giving greater stimulation to the intestinal tract. Eppinger and Hess (7) attribute the diarrhea to the increased activity of the vagus nerve. Crotti (8) says that the stomach of goiter patients is in a state of constant spasticity as observed fluoroscopically. Baker (9) mentions diarrhea as a common

¹ This work has been aided by a grant from the Rockefeller Foundation Biological Fund, University of Chicago.

symptom in exophthalmic goiter. Lockwood (10) finds diarrhea present in only 4.4 per cent of the 90 cases with an average basal metabolic rate of +35 which he studied. Urmössey and Lukacs (11) found that in 15 of 24 cases gastro-intestinal motility of babies was speeded up by treatment with thyroid material. Deusch (12) also finds that thyroglandal injections increase intestinal motility as revealed by the fluoroscope.

Our work includes studies of the effect of thyroid feeding on the motor activity of the gastro-intestinal tract of dogs. We studied the effect of thyroid feeding on the gastric contractions of the empty stomach, and also on the movement of food through the digestive canal.

METHODS. The experiments to be described were run on six dogs. The dogs were kept on a constant diet consisting of 250 grams of meat, 250 cc. of milk, and 200 grams of bread per day. On this diet the dogs maintained their normal weight. The general condition of the dogs was watched, and the weight and temperature were recorded every other day. On one dog basal metabolism tests were frequently taken.

The activity of the gastro-intestinal tract was studied when the stomach was empty by means of the balloon method. The dogs were trained to swallow a stomach tube with a condom balloon attached and the records of the contractions of the empty stomach were taken according to the method suggested by Boldyreff and perfected by Carlson. A bromoform monometer was used, and the pressure at the beginning of each experiment was one centimeter of bromoform. The motility was recorded for a period of three hours, beginning 20 or 24 hours after the dogs had been fed.

The progress of food through the digestive tract was observed by means of the fluoroscope. Once a week, instead of the usual meal, the dogs were fed 200 grams of ground meat mixed with 70 grams of barium sulphate. Note was taken 1, of the time when food first left the stomach; 2, of the time when material entered the ascending colon; 3, of the emptying time of the stomach; 4, of the emptying time of the colon.

The gastric motility was studied by the above methods on the normal dogs for a period varying from two weeks to one month. The dogs were then fed daily 0.4 gram per kilo body weight of Armour's desiccated thyroid in addition to the usual meal. On this amount of thyroid the body temperature rose and the weight decreased. The dog on whom basal metabolism studies were made showed a basal rate increased to +50 per cent. The dogs showed increased nervousness, if restlessness, irritability of temper, and twitchings of the muscles are indications of this condition. Although the water balance was not measured, it was obvious that the dogs drank far more water than normally and urinated more copiously. All the dogs showed an increased desire for food; three showed diarrhea (13). By means of these symptoms it can be seen that the condition in dogs during the feeding of thyroid material resembles spontaneous hyperthy-

roidism in man. The hunger contractions and the progress of the barium meal were studied throughout the period of thyroid feeding. This period varied from one to two months depending on the time necessary for the symptoms to manifest themselves, and their severity.

After thyroid feeding was discontinued, the gastro-intestinal activity was studied for a period of from one to two months.

RESULTS. 1. *Gastric contractions.* In our six dogs, the normal type of gastric contractions varied. Three of these dogs showed low motility, described by Carlson as the 20 second rhythm (14), type 1, or complete rest of the stomach. On the remaining three dogs vigorous type 2 contractions (15) were usually observed.

The gastric motility of all dogs definitely increased over the normal during the period of induced hyperthyroidism. Records of higher activity were obtained about a week after the ingestion of thyroid was initiated. The length of the hunger periods and the height of the individual contractions were increased, also periods of increased tonus became frequent. A vigorous type of activity showing type 2 contractions on which were superimposed smaller contractions of the 20 second rhythm was observed frequently in all dogs during the "hyperthyroid" period.

After the thyroid feeding was discontinued, gastric motility remained high for a few days (average, six) and thereafter dropped. In two dogs the motility steadily decreased to the normal level and remained there. In the other dogs, while the motility decreased below that observed in the hyperthyroid phase, it did not drop to the normal level except occasionally. Usually the contractions were higher than those observed before thyroid was fed, and occasionally high contractions like those observed during the "hyperthyroid" period occurred as long as two months after the thyroid feeding had been stopped.

It is interesting to note that the gastric motility of the dog on which basal metabolism tests were run never reached the normal level during the two months that contractions were recorded following the feeding of thyroid. However, the basal metabolic rate fell from +50 per cent to the normal at the end of the first week after the thyroid feeding was discontinued, and remained there.

2. *The gastro-intestinal activity as observed by the fluoroscopic method.* The progress of the barium meal through the gastro-intestinal tract of the dogs showed a greater uniformity than the contractions of the empty stomach. In four of the dogs the barium meal usually stayed in the stomach until late during the first hour after its ingestion, or during the second hour, when small particles could be observed in the intestines. Food usually reached the ascending colon five hours after feeding. The complete emptying time of the stomach varied between 5 and 10 hours. The colon was not emptied of barium until the next day, about 24 hours

after its ingestion. Two of the dogs showed intestinal rates varying considerably from the above description.

One of these dogs showed a faster normal rate. Barium was always observed in the small intestine within the first hour after feeding. It always reached the ascending colon between the third and fourth hours after feeding. The emptying time of the stomach was variable, occurring between 6 and 10 hours after feeding. The time of defecation was also variable, barium frequently being given off 2 or 3 hours after it had reached the descending colon. The bulk of the material was not expelled until the following day, however.

One dog showed extremely slow progress of the food through the digestive tract. In only one instance out of four observations was barium observed in the small intestine during the first hour after ingestion. It did not reach the ascending colon until 5 or 6 hours later. The stomach normally showed the presence of barium in small amounts 10 to 11 hours after feeding. In one instance only the stomach was completely empty 8 hours after feeding.

It is interesting to note that the dog whose intestinal rate was abnormally fast was one of those animals showing high gastric motility as revealed by the gastric hunger contractions; and that the dog whose intestinal rate was slower than the others showed low gastric hunger contractions during the period previous to thyroid feeding.

During the period of thyroid feeding increased gastro-intestinal motility was shown by means of the fluoroscope in five of the six dogs. Appreciable quantities of barium always appeared in the intestine during the first hour after its ingestion. Barium appeared in the ascending colon 1 to 2 hours earlier than in the normal condition, or 3 to 4 hours after ingestion. While the emptying time of the stomach appeared to be variable according to the method of observation, the stomach was always emptied at least by the shortest time limit observed in the normal condition. In all except one dog, barium remained in the colon for 24 hours after feeding, as before. In the exceptional dog, the bulk of the barium was always in the descending colon in 8 hours and defecation occurred at the tenth hour in five out of six observations. The stools of this dog were always very soft or fluid in consistency during the period of "hyperthyroidism." In two other dogs the stools were softer in consistency than normal during the "hyperthyroid" period, but nevertheless the barium was retained the normal length of time in the colon.

The dog whose intestinal rate has been described as unusually rapid normally showed no appreciable change during the period of induced hyperthyroidism. However, in this dog, the activity of the empty stomach was increased as recorded by the balloon method.

The intestinal rates of the five dogs which increased during the "hyper-

thyroid" period returned to the previous normal level within two weeks after the thyroid feeding was discontinued.

DISCUSSION. Perussé and Rozen (4) report an increase in the height of gastric contractions in dogs fed daily 2.5 grams desiccated thyroid per kilo body weight. When the dose is increased to 5 grams per kilo body weight they note a preliminary period of depression followed by an increase in height of the gastric contractions. With a 15 gram dosage they report no constant increase in hunger contractions.

Kratinoff (5) reports in four of the six dogs in which he studied the effect of "hyperthyroidism" on gastric contractions, an increase in the hunger contractions during the period of thyroid administration, whether the thyroid was given in one single large dose, or fed daily in small amounts, or whether thyroxine was injected. In two of the six dogs, he reports a depression of the hunger contractions under similar conditions.

All of our six dogs showed an increase in hunger contractions during thyroid feeding, but the effect of administering thyroid in varying doses was not studied. The dose used (0.4 gram per kilo body weight) brought on symptoms similar to those seen in the spontaneous hyperthyroidism of man, including an increase in the basal metabolic rate. Increased gastric motor activity was observed to accompany this condition in our animals.

Kratinoff reports that ten months after daily feeding with 1 gram of desiccated thyroid for a period of thirty days, the gastric contractions were often as high as during the "hyperthyroid" period. He finds that the stomach behaved erratically after "hyperthyroidism," at one time showing a depression in motility, and at another time very high contractions. He concludes that the thyroid has a long continued effect on gastric motility, which he did not explain.

We found a decrease in the activity after the thyroid was discontinued, but we occasionally observed the type of contractions, characteristic of the "hyperthyroid" condition. After two months, the gastric activity had returned to the normal level in only two dogs. The explanation of this long continued action of the thyroid on the stomach is difficult. All other hyperthyroid symptoms disappeared. The dogs gained weight, frequently above the normal level. The nervousness, the elevated body temperature decreased, the polydipsia, and polyurea disappeared; and in the dog on whom basal metabolic studies were made, the basal returned to the normal. We cannot assume therefore that any abnormal amount of functioning thyroid material remains incorporated in the protoplasm long after the thyroid intake is stopped. Careful studies on the blood chemistry of dogs, the effect of low protein and cholesterol values on the gastric contractions, and a study of these values in the blood of dogs long after periods of thyroid feeding might throw some light on these conditions.

Not only is the digestive tract shown to be more active during the time of thyroid feeding by the gastric contractions but also by the barium meal. Inasmuch as diarrhea is a frequent symptom of hyperthyroidism in man, one might expect to find that the barium residue would go through the colon at a greater rate of speed than the normal, so that water absorption would be prevented. This expected condition was actually observed in only one of the six dogs studied. In this dog the stools were definitely of more fluid consistency than the normal. In two other dogs softer stools than normal were observed, but in these dogs the barium residue was retained the usual length of time. In the three other dogs the barium remained in the colon the normal length of time and no symptoms of diarrhea were manifested.

In all dogs except one, however, an increased speed of the barium meal through the stomach and small intestine was noted.

Why the increase in speed of the barium meal should occur in the upper part of the digestive tract is difficult to explain. If the thyroid should act by means of the vagus nerve this effect would be expected, but later studies indicate that the thyroid does not act in this manner. Furthermore, the fact that gastric acidity is decreased rather than increased during hyperthyroidism (17), (18), (19) does not indicate over activity of the vagus. McCann (20) has lately investigated the factors which might influence the emptying time of the stomach. He finds that free hydrochloric acid or the products of digestion have no effect on the emptying time. He finds in the first phase of digestion a limited emptying of the stomach accompanied by great tonic and peristaltic activity of the antrum. As the antrum relaxes in the later phases of digestion, the emptying time decreases. McCann thinks that the activity of the antrum depends on the irritability and stimulating action of raw protein to vigorous tonic and peristaltic contractions. He also thinks that the change of the meal to the more fluid state is a factor influencing the emptying time of the stomach. Should one assume then that the thyroid acts by directly stimulating the muscle to greater activity, one might conclude that one should find delayed emptying of the stomach since the higher activity of the antrum tends to keep food within it, according to McCann. However, the contrary is true. Greater activity of the muscles of the intestinal tract would bring about an increased rate of speed with which barium entered the large intestine, and would therefore explain that finding.

Explanation of the increased intestinal activity might lie in the change in gastric secretion. Since gastric acidity is lessened in hyperthyroidism protein food is probably not as well digested as in the normal dog, and larger pieces of undigested food might pass into the small intestine acting as a stimulus to peristaltic activity.

SUMMARY

1. Daily feeding of 0.4 gram per kilo body weight of desiccated thyroid per dog increases the activity of the empty stomach of dogs as shown by the type and height of the contractions of the empty stomach. After the thyroid feeding is discontinued there is a lowering of gastric activity, although in two months a return to the normal level was not observed in two dogs.

2. During the period of thyroid feeding the emptying time of the stomach is decreased, and a barium meal passes more rapidly through the small intestine. After the administration of thyroid is stopped, the speed of the barium meal through the digestive tract returns to its former rate.

BIBLIOGRAPHY

- (1) BOOTHY, W. M. AND I. SANDIFORD. *Journ. Amer. Med. Assoc.*, 1923, lxxxi, 795.
- (2) CARLSON, A. J. *The control of hunger*. University of Chicago Press. 1916, chaps. 3 and 4.
- (3) CARLSON, A. J. *Ibid.*, chap. 16.
- (4) PERUSSÉ AND ROZEN. *This Journal*, 1929, xci, 291.
- (5) KRATINOFF. *Zeitschr. f. d. gesamt. exp. Med.*, 1929, lxiv, 376.
- (6) KING, C. H. *Med. Clin. N. Am.*, 1919, ii, 1655.
- (7) EPPINGER AND HESS. *Zeitschr. klin. Med.*, 1909, lxvii, 231.
- (8) CROTTI, A. *Thyroid and thymus*. Lea & Febriger, Philadelphia, 1918.
- (9) BAKER, L. F. *Trans. Amer. Gastro-Enterol. Assoc.*, 1918, May 7.
- (10) LOCKWOOD. *Journ. Amer. Med. Assoc.*, 1925, lxxxv, 1032.
- (11) URMÖSSY, E. AND J. LUKACS. *Arch. f. Kinderh.*, 1930, lxxxix, 161.
- (12) DEUSCH, G. *Deutsch. Arch. klin. Med.*, 1923, cxlii, 1.
- (13) KUNDE, M. M. *This Journal*, 1927, lxxxii, 195.
- (14) CARLSON, A. J. *Op. cit.*, p. 36.
- (15) CARLSON, A. J. *Ibid.*, p. 44.
- (16) HARDT. *This Journal*, 1916, xl, 314.
- (17) TRUESDELL. *This Journal*, 1927, lxxvi, 20.
- (18) CHANG AND SLOAN. *This Journal*, 1927, lxxx, 732.
- (19) McCANN. *This Journal*, 1929, lxxxix, 497.

II. THE EFFECT OF INDUCED HYPERTHYROIDISM ON THE GASTRO-INTESTINAL MOTILITY OF VAGOTOMIZED DOGS¹

DOROTHY FETTER, LOUIS BARRON AND A. J. CARLSON

From the Physiological Laboratory, University of Chicago

Received for publication May 26, 1932

Previous studies have shown that dogs which are fed daily 0.4 gram per kilo body weight of desiccated thyroid show an increase in the contractions of the empty stomach, and an increase in the speed with which a barium meal passes from the stomach to the colon. Clinical observations and experiments show that the motor activity of the digestive tract is increased during hyperthyroidism. Various explanations have been given for this.

Möbius (1) thinks that the thyroid secretion works directly on the intestinal wall. Wolpe (2) assumes that the lack of free hydrochloric acid observed in many hyperthyroid cases is responsible for the diarrhea of hyperthyroidism. Deusch (3) thinks that the thyroid hormone acts on the nervous mechanism of the intestine, while Eppinger and Hess (4) attribute the diarrhea of hyperthyroidism to overactivity of the vagus nerve.

The following studies were undertaken in order to see whether the vagus nerve is responsible for the increase in the motor activity of the stomach and small intestine which we observed in our previous studies. The anterior and posterior branches of the vagus nerve were sectioned just below the diaphragm in four dogs. The gastric hunger contractions, and the motor activity of the digestive tract as shown fluoroscopically were studied for two months following the vagotomy. Four-tenths gram of desiccated thyroid per kilo body weight was given the dogs daily, and the gastrointestinal activity studied as before. After one to two months the feeding was discontinued and the activity of the digestive tract was studied for two months longer. The methods used for these studies were described in paper I.

To make sure that the vagus nerve was cut and did not regenerate during the time of the experiment, the effect of insulin on the gastric contractions was observed. Insulin increases the gastric contractions of the normal animal (5) and decreases those of the vagotomized dog (6). At the conclusion of the experiment, the effect on the digestive tract of stimulating

¹ This work has been aided by a grant from the Rockefeller Foundation Biological Fund, University of Chicago.

the vagi above the point of section was noted. Contraction of the stomach or intestine on stimulation of the vagi shows that some fibers are functioning. No activity of the gastro-intestinal muscles on stimulation of the vagi indicates that the nerves are sectioned and have not regenerated.

RESULTS. *Dog. 1.* 1. *Gastric contractions.* The contractions of this dog when vagotomized are usually type 1 (7). The interval between contractions varies between two and ten minutes, thereby differing from normal type 1 contractions where the interval is usually about one minute. The type of contraction observed in dog 1 is characteristic of vagotomized dogs (8). Frequently 20 second rhythms (9) are noted with a height of $\frac{1}{2}$ to 1 cm.

For about ten days after the feeding of thyroid had been started, the gastric contractions showed no change from the normal. Then a slight evidence of increasing hunger contractions was observed. Type 1 contractions were seldom noted and the 20 second rhythms predominated. The 20 second rhythm reached 2 or 3 cm. in height with occasional periods of type 2 contractions interrupting.

The "hyperthyroid" type of contractions continued for about a month after thyroid feeding had been stopped; then the normal type 1 contractions became the predominating type observed.

Dog 1 showed by the balloon method a slight increase in gastric activity during the period of thyroid feeding.

2. *The gastro-intestinal activity as observed by the fluoroscopic method.* In four of the seven observations made two months subsequent to the vagotomy, food was not seen in the intestine until three or four hours after its ingestion, a considerably longer time than was ever observed in the unoperated dogs. A small shadow was usually noted in the stomach on the day following the taking of barium. This was never observed in the normal dog. Barium reached the ascending colon six or seven hours after feeding. Defecation of barium occurred at a period varying between twenty-four to thirty-one hours after its ingestion.

After thyroid feeding was initiated a great increase was noted in the speed of the barium meal throughout the digestive tract. Food was always observed in the small intestine at the second hour after feeding. During the first two weeks of thyroid feeding, faint shadows were observed in the stomach twenty-four hours after the ingestion of barium; thereafter the stomach emptied itself within the first ten hours. During the first two weeks of thyroid administration, barium was observed in the ascending colon no earlier than six hours after ingestion. After two weeks of thyroid feeding the dog developed diarrhea. Defecation of barium occurred shortly after it reached the descending colon, and continued to be passed at intervals as more accumulated in the rectum. The last remnant of barium was passed during the night or on the morning after its ingestion.

After thyroid administration ceased, the stomach continued to show the same type of activity as during the "hyperthyroid" phase; the intestinal activity lessened, however. Barium was often observed in the small intestine within the first hour after its ingestion. Readings made twelve hours after the taking of barium always revealed shadows in the stomach, but for seven weeks the stomach was completely empty twenty-four hours after feeding. Food was not observed in the ascending colon until five or six hours after its intake, however, and the diarrhea disappeared. Defecation of normally formed barium stools occurred the morning after the material had been taken.

Dog 1 was operated June 4. The effect of insulin on the hunger contractions was noted August 9 and October 24. In both cases the hunger contractions ceased within an hour after the injection of insulin, and no increase in the contractions was noted while the record was taken. This indicates that the vagus nerves to the stomach were not functioning. On November 8, five months after operation, the vagi were stimulated electrically in the neck, and the effects on the stomach observed. No gastric or intestinal activity occurred on stimulation, therefore we assumed that the fibers of the vagus leading to the digestive tract had been successfully sectioned, and no regeneration had taken place during the course of the experiment.

Dog. 2. After vagotomy dog 2 showed a decided decrease in the gastric contractions as compared to those observed in the normal. The normal type of motility previous to vagotomy had been type 2 (11) rising to a height of $2\frac{1}{2}$ to $5\frac{1}{2}$ cm. After vagotomy, 20 second rhythm rising to a height of only $\frac{1}{2}$ to 1 cm. was frequently observed. Often for three hours only low tonus changes were recorded. Type 1 motility also occurred with the interval between contractions varying between three and ten minutes.

After thyroid feeding was initiated, the 20 second rhythm was still predominant, although it differed from that previously observed by rising in height to 2 or 3 cm. During the second month of thyroid feeding, tonus changes were frequently associated with the 20 second rhythm, and often it was varied by falling into the 20 second rhythm interrupted by ten minute stretches of type 2 contractions. A half-hour or an hour of type 2 contractions frequently interrupted an otherwise low record of motility.

After thyroid feeding was stopped, the motility continued of the same type as we observed during the "hyperthyroid" phase for three weeks. Then activity dropped to the normal level; 20 second rhythm rising to a height of $\frac{1}{2}$ to 1 cm. predominating, with occasional records showing type 1 motility.

After vagotomy, food was observed in the stomach twelve hours after

feeding, and frequently small barium shadows were present twenty-four hours after ingestion. The stomach then retained its contents for a much longer time after vagotomy than before. However, for the first month after the section of the vagi, the intestine showed much greater activity than the normal. Whatever material escaped from the stomach passed through the small intestine quickly, and barium was observed in the ascending colon three hours after feeding. Soon after it reached the descending colon it was expelled, and defecation of barium occurred at intervals of one or two hours until it was completely gone. The stools were fluid. The dog manifested a severe diarrhea constantly during this period. A month after the operation the diarrhea stopped, and food passed more slowly through the entire intestine. It did not reach the ascending colon until four to five hours after ingestion. Defecation occurred eight hours after food intake, and the stools were firmer than previously.

After the intestinal activity had remained at a low rate for three weeks, thyroid feeding was started. The intestinal rate increased. Food reached the ascending colon usually at the third hour after its ingestion. Defecation of barium frequently occurred five hours after feeding, and the diarrhea became severe. No noticeable difference was observed in the activity of the stomach. Some barium was always seen in the stomach twelve hours after feeding, but a residue was observed the next day on only two occasions.

After the thyroid feeding was discontinued the only noticeable difference in the passage of the barium meal was that it remained an increased length of time in the colon. Defecation did not occur as a rule within the first twelve hours after eating. No difference was noted in the motility of the stomach, and the barium reached the ascending colon three or four hours after its ingestion. Four weeks after the thyroid administration was stopped, barium reached the colon four to five hours after feeding, indicating a gradual decrease in the activity of the gastro-intestinal tract.

During the course of the experiment the effect of insulin on the gastric contractions was observed. In no case was there an increase in contractions, and a decrease was observed $\frac{1}{2}$ to 1 hour after the injection of insulin. On May 10, five months after vagotomy, the vagi were stimulated in the neck and the gastric activity recorded. Under light ether anesthesia all activity of the stomach had ceased, and no contractions were noted when the vagi were stimulated with the induction coil. We therefore assumed that the vagus fibers to the stomach were not functioning.

In this vagotomized dog then, the hunger contractions increased during the "hyperthyroid" period. Three weeks after the thyroid feeding was stopped the hunger contractions returned to the type of activity observed during the normal.

The results on the gastro-intestinal activity as revealed by the fluoro-

scope show an increase during the "hyperthyroid" phase over the activity just preceding it. However, the fact that motility similar to that observed while thyroid was being fed occurred subsequent to vagotomy opens the question whether or not thyroid has anything to do with the change in speed of the digestive tract.

Dog. 3. After vagotomy dog 3 showed severe intestinal disturbances. Food remained in the stomach for long periods of time. Often considerable amounts of a barium meal were observed in the stomach forty-eight hours after eating. Consistently with this slow gastric motility the dog lost weight. The weight previous to the vagotomy was 11.7 kilos, a month later it had decreased to 10.5 kilos. If this dog was fed daily it was impossible ever to find a time when the stomach was empty unless vomiting had just occurred. While some records of gastric contractions were made after vomiting, it was considered best not to interfere with such nutrition as the dog could obtain so the study of hunger contractions was not made.

Such records of gastric activity as we obtained, however, showed that either type 1 contractions or very low tonus changes occurred. In order to obtain evidence that the vagi were sectioned the effect of insulin on gastric contractions was studied. Twenty-five minutes after an injection of insulin during a period of type 1 contractions, all activity of the stomach ceased, and was not resumed while the record continued for another hour. The results indicated that the vagi were not functioning.

Studies of the activity of the digestive tract by the fluoroscopic method show low motility following vagotomy. Previous to the operation the emptying time of the stomach had been six to seven hours. After the section of the vagi, the stomach contained considerable amounts of food for two or even three days after ingestion. Barium reached the ascending colon at a time varying between five to eight hours after its intake. Defecation of some barium occurred the day after it had been fed. A month after the operation the fluoroscopic readings showed considerable decrease in the emptying time of the stomach. Some barium was usually found in the stomach twenty-four hours after feeding, but the residue was small and the main bulk of the meal had obviously passed on. Simultaneously with this improvement in gastric activity the dog gained weight and her general condition improved.

When the weight reached 11.6 kilos, thyroid was given (March 24). By April 5 symptoms of severe hyperthyroidism were manifest. The dog's weight had by this time decreased to 11.4, the temperature raised from 98° to 101° F. Polydipsia was very noticeable; the dog's water jar had to be refilled about three times daily. Accompanying the polydipsia was a noticeable polyurea. A severe diarrhea had started. The fluoroscopic observations made on April 5 and April 12 showed that barium entered the ascending colon at the third hour after food intake. Defe-

cation occurred frequently throughout the day, and the gastric intestinal tract was emptied completely about eleven hours after the barium meal had been eaten. In ten days the dog died, apparently from the effect of thyroid feeding. Autopsy revealed no abnormality of the internal organs except a hemorrhagic intestine.

Although this experiment could not be completed, we have included it here because it shows the extreme disturbance in gastric motility which may follow vagotomy. Also the great increase in motility following thyroid feeding is significant.

Dog. 4. 1. Gastric contractions. Shortly after the vagotomy, this dog showed a severe diarrhea which persisted for a month. At the end of that time, the dog recovered, and one month after the dog had been in good health, and his weight constant for two weeks, studies on gastric contractions showed low motility. Either type 1 contractions, occurring every three to five minutes, about 2 to 4 cm. in height were observed, or low 20 second rhythm about $\frac{1}{2}$ to 1 cm. high.

Two weeks after the feeding of thyroid was initiated the gastric contractions increased in height. Increases in tonus were frequently observed. Type 1 contractions occurred often, coming every minute and reaching a height of 12 to 14 cm. After six weeks thyroid feeding was discontinued, and gastric activity as recorded by the balloon method sank to the previous level.

2. The gastro-intestinal activity as observed by the fluoroscopic method. Previous to thyroid feeding, the gastro-intestinal rate as observed by the fluoroscope showed that food had escaped into the small intestine during the first hour. It reached the ascending colon between the fifth and sixth hours. Fragments of barium were observed in the stomach twenty-four hours after its ingestion. The colon was emptied between twenty-four and thirty-six hours after the barium had been given.

During the period of thyroid feeding, barium entered the ascending colon two or three hours after feeding. Fragments were not observed in the stomach the next day. There was no diarrhea; the barium was not expelled from the colon until twenty-four hours had elapsed. The increased motility was observed in the upper part of the gastro-intestinal tract, as before vagotomy. A week after thyroid feeding was discontinued the gastric motility returned to its former level.

The studies on motility were continued for a month after thyroid feeding stopped. Then a study of the gastric motility following electric stimulation of the vagi above the point of section was made. No movement of the stomach or intestine following stimulations could be detected.

DISCUSSION. The changes in the activity of the digestive tract after vagotomy are of interest. A great decrease in the activity of the stomach is shown both by the gastric hunger contractions, and by the length of

time that shadows are observed in the stomach after barium intake. Dog 3 shows that the decrease in gastric motility may reach dangerous limits. Gradually the digestive tract seems to adapt itself to the absence of the motor nerve, however.

The diarrhea following vagotomy which is reported in dogs 2 and 4 is of interest. We have observed this phenomenon in other vagotomized dogs. In two cases food was frequently defecated three hours after intake with very little change in its condition. Particles of meat were observed in the feces. In all dogs this diarrhea ceased after a month. The diarrhea might be due to changes in gastric secretion. Inasmuch as gastric acidity and secretion are lessened by the section of the vagus nerve, we may assume that digestion is impaired in vagotomized dogs. Particles of undigested food then enter the intestine and serve as a stimulus to its greater activity. In time the glands of the stomach may adapt themselves to the absence of the secretory nerve, and digestion proceeds, resulting in a disappearance of the diarrhea.

Since these vagotomized dogs show an increase in gastro-intestinal activity upon the administration of thyroid, it seems clear that the influence of the thyroid on gastro-intestinal motility is largely if not wholly independent of the possible influence of the thyroid hormone on the gastro-intestinal vagus mechanism.

SUMMARY

Upon the administration of thyroid substance, vagotomized dogs show: 1, an increase in hunger contractions; 2, an increase in the speed with which a barium meal passes through the digestive tract, particularly the stomach and the small intestines.

BIBLIOGRAPHY

- (1) MÖBIUS, P. J. *Die Basedow'sche Krankheit*. Vienna, 1906.
- (2) WOLPE, I. M. *Deutseh. Arch. f. klin. Med.*, 1912, cvii, 492.
- (3) DEUSCH. *Die Hyperthyreosen Handh. d. inn. Sekretion von Hirschl.*, 1917, pp. 1-70.
- (4) EPPINGER AND HESS. *Zeitschr. klin. Med.*, 1909, lxxviii, 231.
- (5) BULATAO AND CARLSON. *This Journal*, 1924, lxxviii, 148; lxxix, 107.
- (6) QUIGLEY, J. P. AND R. Q. TEMPLETON. *This Journal*, 1929, xci, 482.
- (7) CARLSON, A. J. *The control of hunger*, p. 44.
- (8) CARLSON, A. J. *This Journal*, 1913, xxxii, 369.
- (9) CARLSON, A. J. *The control of hunger*, p. 36.
- (10) FETTER, D. AND CARLSON, A. J. *This Journal*, 1932, ei, 598.
- (11) CARLSON, A. J. *The control of hunger*, p. 44.

FACTORS WHICH INFLUENCE THE FLOW AND PROTEIN CONTENT OF SUBCUTANEOUS LYMPH IN THE DOG

II. THE EFFECT OF CERTAIN SUBSTANCES WHICH ALTER THE CAPILLARY CIRCULATION¹

FLORENCE W. HAYNES

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication May 27, 1932

Previous observations on the effect on the lymph of vasodilator and vasoconstrictor substances, such as histamine, adrenin and pituitrin, have been made on thoracic duct lymph, and are summarized in table 1. Few, if any, determinations have appeared on lymph coming directly from the subcutaneous areas of the body where the effects of digestion and respiration are relatively unimportant. Recent work in this laboratory (Haynes, 1932; White, Field and Drinker, 1932) has indicated the part played by changes in arterial and venous pressure in the flow and concentration of lymph in the dog. In the present study on subcutaneous lymph, observations have been made of the effects of certain substances of general physiological significance, namely, histamine, acetyl choline, adrenin, ephedrine and posterior pituitary extract. It was hoped, by a comparison of their actions on the small blood vessels, to determine the relative importance of various capillary changes in the production of lymph.

METHOD. Lymph was obtained from young dogs (18 to 31 kilos) under pentobarbital-sodium, "Nembutal" (sodium-ethyl (1-methyl-butyl) barbiturate) anesthesia. The flow from the foot was measured by collecting the lymph from one or two of the main lymphatic trunks at the ankle in calibrated cannulas which were emptied every 10 minutes. Injections of the lymphatics of the dog's leg have shown that cannulation of the two main trunks on the front of the foot represents approximately one-half of the flow of lymph from the paw. In order to obtain a flow of lymph, the feet were moved passively by attaching them to a revolving wheel. Since the dogs were usually on their backs with the feet slightly above heart level, it was thought that the gradual decrease in lymph, sometimes observed for several hours after cannulation, might be partly

¹Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Radcliffe College.

TABLE 1
The effect of histamine, adrenin and pituitrin on thoracic duct lymph

SUBSTANCE	DOSE	OBSERVER	DATE	ANIMAL	LYMPH*		REMARKS
					Flow	Per cent protein or concentration	
Histamine	0.3 mgm. per kilo intravenously	Dale and Laidlaw	1911	Dog	+	Slight +	Anaphylaxis has been found to give the same effect (Petersen and Hughes, 1925a)
	1:1000 clinical adrenalin intravenously	Camus	1904	Dog	+		
Adrenin	Dog, 0.3 mgm. per kilo subcutaneously	Tomaczewski and Wilenko	1908	Dog and rabbit	-		Splanchnic stimulation increases lymph production (Starling, 1894)
	0.04 per cent 9 cc. in 30 min. intravenously	Bainbridge and Trevan	1917	Dog	+	Slight +	
	0.5 to 1.0 cc. of 1:1000 or a dilution	Yanagawa	1916	Dog	+	+	
	2 to 5 cc. 1:10,000 intravenously	Christoni	1921	Dog	+	-	
	1 to 4 mgm. in 10 to 20 cc. saline intravenously	Petersen and Hughes	1925b	Dog	+	-	
Pituitrin		Meyer-Bisch Gunther and Boek	1926	Dog	+	+	
	2 to 4 cc. intravenously	Meyer and Meyer-Bisch	1921	Dog	-	+	
	2 to 3 cc. (Parke, Davis & Co.)	Bayley, Davis, Whiteman and Scott	1925	Dog	-		
	0.5 cc. (Armour) intravenously	Petersen and Hughes	1925b	Dog	-	0	

* A plus indicates an increase and a minus a decrease; a plus followed by a minus indicates an increase and then a decrease.

due to the position of the dog. To avoid confusion of results with pressor substances, after which a fall in lymph flow might be expected, in a few experiments animals were placed either in the feet down position or on the side. The skin temperature was followed by a thermocouple on the shaved surface of the foot.

Observations on subcutaneous lymph from the head and neck region have been omitted since the flow of cervical lymph, as that of thoracic duct lymph, was found to follow the mechanical effects of respiratory changes much more closely than circulatory changes.

Injections were made into the cannulated jugular vein or into the femoral artery through a small cannulated arterial branch, and the blood pressure recorded from the carotid artery. At intervals samples of arterial blood were taken for hemoglobin (Sahli hemoglobinometer) and hematocrit readings. The protein content of the lymph and serum was determined refractometrically.

RESULTS. *Histamine.* "Ergamine" acid phosphate (Burroughs, Wellcome & Co.) was injected into the blood stream in doses of 0.39 to 0.88 mgm. per kilogram, or approximately 0.026 to 0.093 mgm. per kilogram per minute, usually in a dilution of 1:1000 in saline. These doses are considerably greater than those found by Koessler and Hanke (1924) to give a perceptible fall in blood pressure in dogs (0.0027 mgm. per minute per kgm. body weight) or those used by Weiss, Robb and Ellis (1932) on humans (0.07 mgm. or 0.001 mgm. per kgm. in a single rapid intravenous injection). Although in all experiments of the present series the blood pressure fell markedly and the rate of respiration was usually increased, in only one case in which the injection was exceptionally rapid did the animal go into shock so that artificial respiration was necessary.

The lymph flow from the foot as well as the protein content of the lymph increased after sufficient doses of histamine given intravenously or when the solution was introduced directly into a branch of the femoral artery. The increase in the lymph flow was almost immediate, appearing in certain cases within two minutes after the beginning of the injection, a significant indication of the speed with which substances pass from the blood into the lymph. Although the lymph flow could be maintained at a high level only as long as injection was continued, the fact that the protein content of the lymph tended to remain above the normal level for some time as well as the appearance of red cells in the lymph in some experiments gave evidence of injury to the capillary endothelium. The change in permeability thus produced apparently has more effect on the lymph than the mere change in diameter of the capillaries.

A few measurements made of the relative amount of plasma, the percent of hemoglobin in the blood, and the concentration of the serum agree with observations in the literature that in anesthetized cats and dogs the

blood is concentrated with a loss of plasma volume after histamine (Dale and Laidlaw, 1919), and that the per cent of serum protein remains practically unchanged or falls slightly (Derer and Steffanutti, 1930).

Acetyl choline. Acetyl choline bromide (Eastman) was used in 1:200 to 1:4000 solution in doses of 0.83 to 6.8 mgm. per kilo, or approximately 0.066 to 0.42 mgm. per kgm. per minute. Injections into the jugular vein in the dog lowered the blood pressure as well as often giving indications of generalized parasympathetic stimulation such as cardiac inhibition, defecation and salivation. The skin temperature of the feet, however, fell slightly instead of rising as might be expected if the arterioles were dilated. The lymph flow decreased, if anything, and the lymph protein remained practically unchanged.

Since it is known that the action of acetyl choline is transient, it was thought that it might be destroyed before it could produce its usual vasodilator effect on the small blood vessels of the foot. For this reason injections were made directly into a cannulated branch of the femoral artery. Control experiments showed that the introduction of saline equivalent in volume to the solution used did not affect the lymph flow. Under these conditions, after acetyl choline, the skin temperature increased indicating arteriolar dilatation. The flow and concentration of lymph showed a moderate but definite increase. In no experiment was there any definite change in the per cent of protein in the serum, nor the per cent of plasma or hemoglobin in the blood.

A typical experiment is seen in figure 1. After the injection of 8 cc. of a 1:200 solution of acetyl choline into the femoral artery, the lymph flow and lymph protein were temporarily increased and the skin temperature rose sharply. One and one-half hours after the injection of acetyl choline, 8 cc. of a 1:1000 solution of histamine were similarly injected. It was assumed that since the lymph flow had returned to normal the acetyl choline had not caused permanent injury but had merely dilated the arterioles. Histamine, which dilates the capillaries and probably also injures their endothelium, caused a much greater flow of lymph and an increase in the lymph protein. This experiment would indicate, as did those of Burn and Dale (1926), that the effect of histamine on the blood vessels is more intense and persistent than that of acetyl choline.

Adrenin. Adrenin was injected at the rate of 0.0012 to 0.0045 mgm. per kgm. per minute, doses which in most cases are within or below the range of 0.0032 to 0.0037 mgm. per kilo per minute, found by Cannon and Rapport (1921) to be the rate of reflex adrenal secretion. Such injections caused in all cases a marked fall in skin temperature due to arteriolar constriction, and when injections were made intravenously a considerable rise in blood pressure. The lymph flow showed a small temporary rise usually followed by a fall, whereas the lymph protein remained practically constant.

An increase of hemoglobin in the blood and of the protein content of the serum as well as a decrease in the relative amount of plasma, observed in a few representative experiments of this series, agree with the reduction in plasma volume obtained by Nelson and Edmunds (1924) after adrenin.

Figure 2 shows graphically the results of a typical experiment of this series. Adrenin in 1:10,000 solution was injected into a cannulated

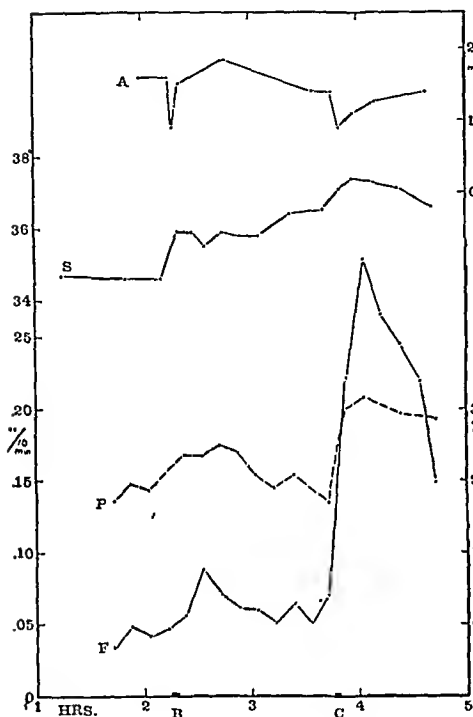


Fig. 1

Fig. 1. The effect of acetyl choline and histamine on the arterial blood pressure, skin temperature, lymph flow and protein content of leg lymph in the dog. *A*, carotid blood pressure in millimeters of mercury; *S*, skin temperature in degrees centigrade; *P*, per cent protein in lymph; *F*, flow of lymph in cubic centimeters per 10 minutes. At *B*, 8 cc. of 1:200 solution of acetyl choline were injected into the femoral artery; and at *C*, 8 cc. of a 1:1000 solution of histamine.

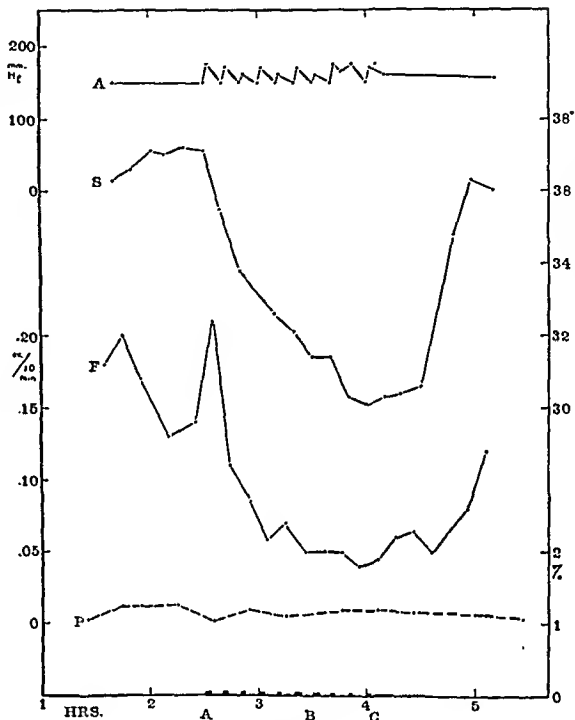


Fig. 2

Fig. 2. The effect of adrenin on the arterial blood pressure, skin temperature, lymph flow and protein content of leg lymph in the dog. *A*, carotid blood pressure in millimeters of mercury; *S*, skin temperature in degrees centigrade; *F*, flow of lymph in cubic centimeters per 10 minutes; *P*, per cent protein in lymph. Lymph was obtained from the left hind leg. From *A* to *B*, at intervals indicated on the abscissa, 18 cc. of a 1:10,000 solution of adrenin was injected into the femoral artery of the left leg; and from *B* to *C*, 12 cc. were similarly injected into the right leg.

branch of the femoral artery. The lymph flow displays a slight initial rise and then a fall as the skin temperature decreases. When the temperature again rises after the effect of adrenin is over, the lymph flow again

returns to normal. The concentration of the lymph did not change, whereas the blood gave evidence of concentration.

Ephedrine. In a few experiments, ephedrine sulphate (3 per cent) was injected into the blood stream in doses of 3 to 16 mgm. per kilogram. The results are similar to those obtained with adrenin, namely, a fall in skin temperature indicating arteriolar constriction, and a temporary rise followed by a late fall in lymph flow with no marked change in the concentration of the lymph. Hematocrit and hemoglobin determinations often indicated a concentration of the blood after ephedrine.

Pitressin. The pressor fraction of the posterior pituitary extract² was used in a few experiments in doses of 2.0 to 7.4 pressor units per kilogram. A decrease in lymph flow usually occurred with the fall which took place in skin temperature, but the effect was less marked than that after adrenin and ephedrine.

DISCUSSION. Table 2 is a summary of all the results reported. In a separate column have been added the effects on the blood vessels recorded by other observers in order that changes in the blood and lymph may be correlated with those in the capillary circulation.

Depressor substances, as histamine and acetyl choline, increase the flow and concentration of subcutaneous lymph. In the dog the change from constrictor to dilator action after histamine takes place at the level of the small visible arteries (Burn and Dale, 1926). In contrast to acetyl choline, histamine produces dilatation of the capillaries of the dog (Abel and Geiling, 1924; Kolls and Geiling, 1924). As in man (Ellis and Weiss, 1932), more profound alterations have been found after histamine than after acetyl choline.

The pressor substances used, as adrenin, pitressin and ephedrine, have been found in general to decrease the flow of lymph without affecting its protein content. In many cases the changes in lymph flow have been found to parallel the changes in skin temperature. It might thus be concluded that a decreased flow of blood through a part, as after the injection of adrenin, is followed by less filtration into the tissue spaces and less lymph. The pressure relationships due to constriction and dilatation of arterioles or capillaries is well shown in a chart by Evans (1930). After adrenin as after histamine and acetyl choline the capillary pressure is lower than the normal. It thus appears that the change in lymph flow after these substances does not follow the capillary blood pressure but is more dependent on the state of the capillary endothelium.

² Pitressin containing 10 pressor units per cubic centimeter was generously supplied by Parke, Davis & Co.

TABLE 2
*The effect on subcutaneous lymph of certain substances which alter the capillary circulation**

SUBSTANCE	NUMBER OF DETERMINATIONS	LYMPH		SKIN TEMPERATURE	BLOOD PRESSURE	CONCENTRATION OF THE BLOOD	EFFECT ON SMALL BLOOD VESSELS	REMARKS
		Flow	Per cent protein					
Histamine	8	+	+	Slight + when injected into femoral artery	-	Hemoglobin + and per cent plasma—. Concentration of serum practically unchanged (Dale and Laidlaw, 1919; Derer and Steffanutti, 1930)	In dogs arterioles and capillaries dilated (Burn and Dale, 1926). Increased capillary permeability	Low body temp. Greater gland secretion. Rapidly destroyed. Possible existence normally in cells for regulation of circulation
Acetyl choline (into femoral artery—irregular or opposite effect intravenously)	4	+	Slight +	+	- +	No definite change in the present experiments	Arteries dilated in cat (Dale and Richards, 1918). Dilatation of cutaneous vessels; action prevented by atropine (Hunt, 1917)	Vagus-like inhibition of heart. Increased gastric secretion. Rapidly destroyed. Probably liberated by parasympathetic stimulation
Adrenin	7	+ -	0	-	+	Per cent hemoglobin + and per cent plasma —, (Nelson and Edmunds, 1924)	Constricts mainly arterioles (Krogh, 1929)	Mobilization of red cells
Ephedrine	3	+ Late fall	0	-	Prolonged +	Per cent plasma usually —, in the present experiments. Occasionally caused hemolysis	Vasoconstriction. Action similar to adrenin (Kreitmaier, 1927)	Increased salivation

Posterior pituitary extract	6	Probably late fall	0	—	+ — waves	Doubtful + in percent hemoglobin (Kolls and Geiling 1924). No change in blood concentration (Bayley, et al., 1925). Dilution of plasma (Himwich, 1932)	In the unanesthetized dog arterioles and capillaries constrict (Kolls and Geiling, 1924)	Diuretic antidiuretic effect. Increased peristalsis
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* A plus indicates an increase and a minus a decrease; a plus followed by a minus indicates an increase and then a decrease.

SUMMARY

1. The vasodilator substances, histamine and acetyl choline, increase the flow and protein content of subcutaneous lymph in the dog. The action of histamine is much more pronounced than that of acetyl choline.

2. Vasoconstrictor substances, as adrenin and ephedrine, cause an increase followed by a decrease in the flow of lymph. Pitressin has less effect but may decrease the lymph flow. The concentration of the lymph remains unchanged after these substances.

The writer wishes to express her thanks to Dr. C. K. Drinker for his interest and helpful suggestions in carrying out this work.

BIBLIOGRAPHY

- ABEL, J. J. AND E. M. K. GEILING. 1924. *Journ. Pharm. Exper. Therap.*, xxiii, 1.
- BAINBRIDGE, F. A. AND J. W. TREVAN. 1917. *Journ. Physiol.*, li, 460.
- BAYLEY, E. C., J. C. DAVIS, W. WHITMAN AND F. H. SCOTT. 1925. *Proc. Soc. Exper. Biol. and Med.*, xxii, 312.
- BURN, J. H. AND H. H. DALE. 1926. *Journ. Physiol.*, lxi, 185.
- CAMUS, L. 1904. *Compt. rend. Soc. de biol.*, lvi, 552.
- CANNON, W. B. AND D. RAPPORT. 1921. *This Journal*, lviii, 308.
- CHRISTONI, A. 1921. *Arch. di fisiol.*, xix, 101.
- DALE, H. H. AND P. P. LAIDLAW. 1911. *Journ. Physiol.*, xliii, 182.
1919. *Journ. Physiol.*, lii, 355.
- DALE, H. H. AND A. N. RICHARDS. 1918. *Journ. Physiol.*, lii, 110.
- DERER, L. AND P. STEFFANUTTI. 1930. *Biochem. Zeitschr.*, ccxxiii, 408.
- ELLIS, L. B. AND S. WEISS. 1932. *Journ. Pharm. Exper. Therap.*, xlv, 235.
- EVANS, C. L. 1930. *Recent advances in physiology*. P. Blakiston's Son & Co., Inc., Philadelphia. See page 73.
- HAYNES, F. W. 1932. *This Journal*, ci, 223.
- HIMWICH, H. E. (To be published.)
- HUNT, R. 1917. *This Journal*, xlv, 197.
- KOESSLER, K. K. AND M. T. HANKE. 1924. *Journ. Biol. Chem.*, lix, 889.
- KOLLS, A. C. AND E. M. GEILING. 1924. *Journ. Pharm. Exper. Therap.*, xxiv, 67.
- KREITMAIR, H. 1927. *Arch. f. exper. Path. u. Pharm.*, cxx, 189.
- KROGH, A. 1929. *The anatomy and physiology of capillaries*. Yale University Press, New Haven. See page 179.
- MEYER, E. AND R. MEYER-BISCH. 1921. *Deutsch. Arch. f. klin. Med.*, cxxxvii, 225.
- MEYER-BISCH, R., F. GUNTHER AND D. BOCK. 1926. *Pflüger's Arch.*, cxxi, 341.
- NELSON, E. E. AND C. W. EDMUNDS. 1924. *Journ. Pharm. Exper. Therap.*, xxiii, 154.
- PETERSEN, W. F. AND T. P. HUGHES. 1925a. *Journ. Biol. Chem.*, lxiii, 179.
- 1925b. *Journ. Biol. Chem.*, lxvi, 229.
- STARLING, E. H. 1894. *Journ. Physiol.*, xvi, 224.
- TOMACZEWSKI, Z. AND G. G. WILENKO. 1908. *Berl. klin. Wochenschr.*, xlv, 1221.
- WEISS, S., G. P. ROBB AND L. B. ELLIS. 1932. *Arch. Int. Med.*, xlix, 360.
- WHITE, J. C., M. E. FIELD AND C. K. DRINKER. 1932. Unpublished.
- YANAGAWA, H. 1916. *Journ. Pharm. Exper. Therap.*, ix, 75.

GLYCOGENESIS IN THE TOTALLY PHLORHIZINIZED ORGANISM

S. G. MAJOR

*From the Division of Experimental Surgery and Pathology, The Mayo Foundation,
Rochester, Minnesota*

Received for publication May 28, 1932

In recent years considerable confusion has arisen in regard to the action of phlorhizin on metabolism of carbohydrate. Difference of opinion still exists as to whether the substance impairs the ability of the organism to oxidize carbohydrate, or whether excretion of sugar is dependent entirely on lowered renal threshold. In the former case, phlorhizin diabetes would be comparable with the pancreatic type, whereas in the latter it would not.

It was thought that a comparison of the glycogenetic ability of the phlorhizinized and depancreatized organisms might throw some light on the question of a comparable defect in carbohydrate metabolism, or of the two processes being due to fundamentally different mechanisms. The results of the studies on phlorhizinized animals are herewith presented. The work on depancreatized animals will be reported subsequently.

LITERATURE. Since so much work has been done on phlorhizin diabetes only those investigations dealing primarily with glycogenesis in the phlorhizinized animal will be mentioned.

Nash found the average glycogen content of the livers of phlorhizinized dogs to be higher than that of dogs of the control series. Palmer demonstrated appreciable increase in both muscle and liver glycogen in one of two dogs after preliminary total depletion of glycogen by the combination of epinephrine and phlorhizin. Paulesco and Michalesco lowered the glycogen content of their phlorhizinized animals by fasting, and two days subsequent to oral administration of glucose were able to demonstrate large quantities of liver glycogen. Sato found that phlorhization does not abolish synthesis of glycogen in the livers of intact rabbits during infusion of glucose. Török showed that six days after administration of phlorhizin had been discontinued, and the animal had been placed on a diet of ox flesh and potatoes, the liver and muscle glycogen of the phlorhizinized dogs was unusually high. Junkersdorf found that in phlorhizinized dogs the content of glycogen of both liver and skeletal muscle was less among those animals that were killed seven hours after administration of the substance had been discontinued, than in those animals that were

killed seventeen hours later. Cori has suggested that the sugar given to a phlorhizinized animal may be temporarily stored as glycogen, even though it may be subsequently recovered quantitatively in the urine. Ringer, Dubin and Frankel expressed the belief that phlorhizinized dogs were able to synthesize glycogen from such glycogenic substances as glycine, propionic acid and lactic acid.

Csonka found no increase in the glycogen content of either liver or muscle among phlorhizinized dogs killed after administration of glucose. Nash and Benedict found that in only one of twelve phlorhizinized dogs was the content of muscle glycogen higher than that of the control series, although in another series of experiments Nash demonstrated that muscle glycogen had been formed in such animals during administration of glucose.

METHODS AND MATERIAL. The dogs used in the experiments were phlorhizinized in the following manner. On the first day of phlorhization 1 gram of the substance, dissolved in solution of sodium carbonate, and 1 gram suspended in olive oil, were injected subcutaneously. Subsequently the animal received 1 gram suspended in olive oil daily for five days. Food was not administered in this period. The glucose-nitrogen ratio was determined in order to demonstrate whether or not the dogs were in the diabetic state before beginning the experiment. In one experiment epinephrine was administered as a measure of depleting glycogen, but this was found to be superfluous because the fasting and ether anesthesia were found sufficient to reduce the glycogen in the muscle and liver to a satisfactorily low value. The specimens were removed either with the animal under the influence of ether anesthesia or after the animal had been killed. Control specimens were obtained in all cases preliminary to the injections of glucose. In the case of the skeletal muscles, control specimens were taken from corresponding muscles of opposite sides of the body. That such muscles can be used as controls had been amply confirmed. The determinations of glycogen were made according to a modification of Pflüger's method and the usual precautions in making determinations of glycogen in tissue were rigidly adhered to. A representative experiment follows.

Experiment 1. February 11, 1931. The dog used in this experiment weighed 10.8 kgm. After phlorhization the first set of specimens of muscle and liver was removed in the usual manner, under ether anesthesia, and employing sterile technic; the second set was removed immediately after the animal had been killed. In the interval of twenty hours between taking of specimens, 1 gram of glucose for each kilogram of body weight each hour was injected intravenously. The glucose-nitrogen ratio before beginning the experiment was 3.62 to 1. The values for glycogen are given in table 1.

COMMENT. Much of the work that has been done on the problem of glycogenesis in phlorhizinized animals is open to criticism in that the experiments have not been adequately controlled. Many investigators have used one series of animals as controls and another series for the actual procedure in question. As is evident from a study of the values for glycogen in the various experiments, different animals of the same species vary widely in glycogen content of both liver and skeletal muscle. Not only do different animals exhibit this variation, but different muscles of the same animal differ widely in glycogen content. Curiously, corresponding muscles of opposite sides of the body of the normal, intact animal conform very closely in the amount of glycogen contained therein. For con-

TABLE 1
Values for glycogen in experiment 1

TISSUE	TIME	DATE	PER CENT GLYCOGEN
	<i>a.m.</i>		
Liver.....	9:00	Feb. 11	0.123
	5:00	Feb. 12	0.750
Left quadriciceps muscle.....	9:03	Feb. 11	0.080
Right quadriciceps muscle.....	5:06	Feb. 12	0.536
Left gracilis muscle.....	9:04	Feb. 11	0.066
Right gracilis muscle.....	5:07	Feb. 12	0.354
Left adductor muscle.....	9:05	Feb. 11	0.154
Right adductor muscle.....	5:08	Feb. 12	0.756
Left sartorius muscle.....	9:02	Feb. 11	0.095
Right sartorius muscle.....	5:07	Feb. 12	0.433

trol material, either the corresponding muscle, or part of the same muscle must be used. In the latter case, care must be exercised to preserve the blood supply. In such a case the distal part of the muscle is used as the first specimen and the proximal part as the final specimen.

In all experiments there was appreciable increase in glycogen content of the liver subsequent to administration of glucose, whereas in the skeletal muscles the glycogenesis was less marked. In five of the seven experiments, however, there was definite evidence of formation of glycogen in muscle.

It is evident that glycogenesis proceeds at a more rapid rate in the liver than in skeletal muscle of the phlorhizinized dog, but as compared with control experiments considerably less glycogen is formed in both the

liver and skeletal muscles of the phlorhizinized dog than in the normal animal under similar conditions.

Although this work cannot answer the question as to whether phlorhizin affects only the renal tissues, or whether it effects a general change in the process of metabolism of carbohydrate, nevertheless it does show that the totally phlorhizinized organism is capable of formation of glycogen.

SUMMARY

A series of seven experiments was performed in which phlorhizinized dogs received intravenous injections of glucose over varying intervals of time. Determinations of glycogen were made on both liver and skeletal muscle before and after injections of glucose. In all cases there was a significant increase in liver glycogen, whereas in five experiments the glycogen content of the skeletal muscle was also definitely increased.

From the data herewith presented it is evident that the totally phlorhizinized dog is capable of glycogenesis in both liver and skeletal muscle.

BIBLIOGRAPHY

- CORI, C. F. 1925. *Journ. Pharm. Exper. Therap.*, xxv, 1.
CSONKA, F. A. 1916. *Journ. Biol. Chem.*, xxvi, 93.
JUNKERSDORF, P. 1922. *Pflüger's Arch.*, cxcvii, 500.
NASH, T. P., JR. 1929. *Journ. Biol. Chem.*, lxxxiii, 139.
NASH, T. P., JR. AND S. R. BENEDICT. 1923. *Journ. Biol. Chem.*, lv, 757.
PALMER, W. W. 1917. *Journ. Biol. Chem.*, xxx, 79.
PAULESCO, N. AND C. MICHAILESCO. 1920. *Compt. rend. Soc. de biol.*, lxxxiii, 566.
RINGER, A. I., H. DUBIN AND F. H. FRANKEL. 1921-1922. *Proc. Soc. Exper. Biol. and Med.*, xix, 92.
SATO, K. 1923. *Tohoku Journ. Exper. Med.*, iv, 347.
TÖRÖK, P. 1924. *Pflüger's Arch.*, cciv, 127.

THE EXCRETION OF URINE IN THE DOG

IV. THE EFFECT OF MAINTENANCE DIET, FEEDING, ETC., UPON THE QUANTITY OF GLOMERULAR FILTRATE

JAMES A. SHANNON, NORMAN JOLLIFFE AND HOMER W. SMITH

*From the Department of Physiology, University and Bellevue Hospital Medical College,
New York City*

Received for publication May 28, 1932

This paper deals primarily with the immediate effects of feeding, and of maintenance diets, upon the glomerular clearance in a single dog, using the excretion of xylose or sucrose as a means of determining the glomerular clearance as described by Jolliffe, Shannon and Smith (1932). (By glomerular clearance we refer to the volume of glomerular filtrate, as measured by the excretion of xylose or sucrose, in cubic centimeters of filtrate per square meter of body surface per minute.) The data are confined to experiments in which the rate of urine flow is above the augmentation limit for urea (Jolliffe and Smith, 1931a, b).

Jolliffe and Smith (1931a) have observed that the post-absorptive urea clearance in dogs is profoundly modified by the maintenance diet; when dog 18 was placed upon a daily ration of cracker meal, 100 grams, sucrose, 30 grams, and lard, 30 grams, the urea clearance fell within a few days from a range of 60-70 to 30, rising again (though not invariably) to 70 or more when the meat diet was resumed. In the following experiments the post-absorptive glomerular clearance in a single female collie (no. 36) has been followed first on a cracker meal diet and then on a meat diet, and observations have been made upon the immediate effects (post-prandial) of eating meat.

The observations begin with experiment 106, at which time the dog had been on a cracker meal diet for 22 days, and the post-absorptive glomerular clearance ranged somewhat below 50, varying slightly, as it usually does, from hour to hour. After a week more on the cracker meal diet the post-absorptive glomerular clearance was close to 40 (expt. 107), falling to 37 in the fifth half-hourly period of observation. The dog was then fed raw beef, and the glomerular clearance began to rise and at the end of five hours reached 74.8, which is an increase of about 100 per cent. The dog was continued on a meat diet for four days (expt. 110) when the post-absorptive glomerular clearance was low again (43.5-51.0); after a meat meal it rose to 75.0.

After two days additional on meat (expt. 111) the post-absorptive glo-

merular clearance ranged from 80.5 to 64.5; after a meat meal it rose to 105.0. Two days additional on meat (expt. 112) produced a post-absorptive clearance of 83.8 rising to a maximum level of 135.0 after meat. Thus in 8 days on a meat diet the post-absorptive glomerular clearance was observed to vary from a minimum of 37.1 to a maximum 83.8, and the post-prandial glomerular clearance was observed to reach a maximum of 135.0. This represents an increase of well over 300 per cent, most of which, we believe, represents a functional variation in glomerular activity.

Three days later, or after a total of 11 days on a meat diet, and after the dog had been fed meat twice on the previous day, the post-absorptive glomerular clearance ranged from 105 to 91, which contrasts with the lower and corresponding level in a single period of 83.8 three days before; after a second administration of xylose and water (but without meat) the clearance fell to 85. (This fall we are inclined to attribute to the additional diuresis resulting from the second administration of xylose and water, because we have observed that in general the glomerular clearance tends to fall during prolonged diuresis when the clearance is not at a basal level.)

The dog was then retired to a mixed diet (predominantly meat) for 20 days when she was again examined to determine the glomerular clearance at low rates of urine flow. Sucrose instead of xylose was used to measure the glomerular clearance in subsequent experiments because it exerts less osmotic pressure in the urine, and hence less diuretic action, at concentrations in the plasma suitable for quantitative determination. The glomerular clearance after a meal in experiment 122 (table 2) ranged from 112 to 138. Seven days later, after 4 days of fasting and 2 days on the cracker meal diet, the post-absorptive glomerular clearance ranged from 80 to 93 (expt. 125); and the next day from 66 to 79 (expt. 126).

The dog was then retired on a mixed (predominantly meat) diet again for a month and finally put upon a cracker meal diet for two weeks. She was given no water for the last three days. The post-absorptive glomerular clearance was observed to range from 38.5 to 52.8 (expt. 137) as when first examined. After three weeks more on the cracker meal diet the glomerular clearance as determined with xylose instead of sucrose ranged from 45 to 59 (expt. 144, table 1). After one week on a meat diet (expt. 147, which is not reported in full here) the post-prandial clearance rose to 106.5, an increase of 235 per cent.

In experiment 151 we have examined the post-prandial effects of eating cracker meal, butter and sugar, in another dog. The glomerular clearance during the pre-prandial period was about 72.0; it remained unchanged immediately after eating, but 5 hours later rose during a single, terminal period to 78.8. This figure may not have been the maximum, but when this result is compared with our previous experiments it appears that the

above food mixture has only a relatively slight action upon glomerular activity. Meat under these same conditions might be expected to raise the glomerular clearance above 100 (cf. expts. 112 and 113). It must be noted, however, that the above food mixture contains nearly 10 grams of protein which itself may account for the observed increase in glomerular activity.

MacKay, MacKay and Addis (1928) and MacKay and MacKay (1931) have shown that protein feeding leads to hypertrophy of the kidneys in the growing rat, and MacKay (1932) implies that the changes in the urea clearance of the dog on mixed as compared to cracker meal diets (Jolliffe and Smith, 1931a, b) are attributable in part to renal hypertrophy. We do not believe that our present results can be wholly explained by hypertrophy; we recognize that it is quite probable that protein feeding in the dog will lead to hypertrophy of the kidneys, as in the rat, but the changes in glomerular clearance which we have described are in adult dogs, and are too rapid and too reversible to be wholly due to hypertrophy. The glomerular clearance was increased from 37.1 to 74.8 in 5 hours (expt. 107); and four days later from 43.3 to 75.2 in the same period of time (expt. 110); after experiment 122 in which the post-absorptive glomerular clearance ranged from 112.0 to 138.3, it fell in eight days of fasting to a low value of 66 (expt. 126). We cannot exclude some hypertrophy and atrophy in our experiments, but certainly we cannot account for such large changes in such a short period of time exclusively on this basis.

Nevertheless this phenomenon is a peculiarly erratic one; the effect of meat tends to persist for some hours after meat feeding so that the effects of a continuous meat diet are cumulative; while on the other hand, the glomerular clearance when elevated by meat is very irregular and tends to drop abruptly to intermediate levels, particularly after prolonged diuresis. This instability at high levels confirms the opinion of Jolliffe and Smith (1931b) that a more uniform clearance may be obtained at low levels (i.e., on a cracker meal diet). Our experience has been that animals reduced to the lower level of glomerular clearance will show (particularly during and after diuresis) an astonishing constancy in behavior, whereas little uniformity can be expected at the elevated glomerular clearance levels observed on a mixed or a meat diet.

The effect of diet upon the $\frac{\text{urea clearance}}{\text{glomerular clearance}}$ ratio. It has been our experience so far that the ratio of the urea clearance to the glomerular clearance may vary considerably in different dogs, though we are not prepared to discuss this point further at this time. We have, however, followed the urea clearance on dog 36 throughout the above experiments because we felt that valid conclusions on the effect of diet could be drawn, at least at the present time, only in this way.

It will be noted that a change of ± 5.0 in empirical urea and xylose clear-

ances of 70 and 100, respectively, produces a change in the ratio of these clearances from 0.62 to 0.74. We believe that this variation is perhaps the magnitude which should be allowed in the above observations for experimental errors (analytical, failure to obtain representative blood or urine samples, or failure to obtain sufficiently flat blood plateaus of sugar, etc.) and consequently a difference in the urea:sugar ratio of ± 0.05 or less is hardly significant.

With this fact in mind it would appear that the urea:sugar ratio is not significantly affected by changing from a cracker meal diet to a meat diet, but remains between 0.65 and 0.75 (see expt. 106 as compared with expts. 110, 111, and 112); nor is this ratio significantly affected by the ingestion and metabolism of meat (expt. 107 and 110).

The effect of the rate of urine flow and of the urea concentration on the urea clearance: glomerular clearance ratio. Jolliffe and Smith (1931a, b) observed an *augmentation limit* for urea (cf. Austin, Stillman and Van Slyke, 1921) at a urine flow of about 0.2 cc. per minute in dogs maintained on a cracker meal diet, and at about 0.4 cc. in dogs maintained on a mixed diet. We cannot discuss at this time the glomerular clearance at urine flows below the augmentation limit, but we must note that at urine flows above 1.0 cc. per minute the rate of urine flow has no effect upon the urea:sugar ratio (expts. 106, 107, 110, 111 and 112) although the concentration of urea in the urine varies from 60 to 2375 mgm. per cent.

In the above experiments the concentration of urea in the plasma varied from 10 to 80 mgm. per cent with no effect upon the urea:sugar ratio.

In experiment 145 (table 1) urea was administered by stomach after the glomerular clearance had been reduced to a low level by maintenance on a cracker meal diet. In three control periods the urea:sugar ratio averaged 0.681; after the urea was administered, this ratio averaged 0.706. Thus no significant change in the urea:glomerular ratio occurred although the urine urea increased from 211 to 3330 mgm. per cent and the plasma urea from 16.4 to 209.2 mgm. per cent.

Thus, under the conditions described above, the urea:sugar ratio is independent of rate of urine flow, blood urea and urine urea concentration. This fact must ultimately be taken into account in considering the question of why the urea clearance is less than the glomerular clearance.

On the other hand, at rates of urine flow below 1.0 cc. per minute there is a significant tendency for the urea:sugar ratio to fall, as shown in experiment 144 with xylose and experiments 122, 125, 126 and 137 with sucrose. This fact suggests the possible passive reabsorption of urea, as was first argued by Rehberg (1926) from a comparison of the rates of excretion of urea and creatinine. But since the present data are not wholly suitable for the examination of this difficult question and since we believe that the problem is open to more direct experimental investigation, we will not dis-

TABLE 1
Effect of diet upon glomerular clearance
(All experiments except nos. 145 and 151 on dog 36)

PERIOD	TOTAL CON- CURRENT TIME	URINE VOLUME PER MINUTE, Y	UREA		XYLOSE		$\text{CM.} = \frac{\text{UY}}{\text{P}} / \text{S.A.}$		CM. UREA CM. XYLOSE
			Plasma	Urino	Plasma	Urino	Urea	Xyloso	
Experiment 106. December 23									
		cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	22	6.09	14.2	57.6	195	1,115	34.3	48.4	0.708
2	45	6.47	14.0	54.5	210	1,186	35.0	50.8	0.689
3	74	5.03	13.5	61.6	234	1,565	31.9	46.7	0.683
4	106	5.79	13.1	55.6	254	1,536	34.1	48.7	0.700
5	136	4.80	12.8	62.9	270	1,866	32.7	46.1	0.709
6	154	4.27	12.6	62.5	286	2,048	29.3	42.5	0.690
(203)	†	See note A. Xylose given <i>per os</i>							
7	240	3.22	11.5	99.7	333	3,895	38.8	52.4	0.740
8	270	2.50	11.1	94.4	338	4,000	29.6	41.1	0.720
9	318	3.15	10.7	100.4	336	4,080	41.0	53.1	0.772
(348)	†	See note B. Xylose given <i>per os</i>							
10	373	2.24	10.2	104.3	352	4,730	31.8	41.8	0.761
Experiment 107. December 30									
1	31	1.90	11.8	135.1	194	3,380	30.4	46.0	0.661
2	60	2.58	11.8	90.0	224	2,642	27.3	42.3	0.646
3	91	3.61	11.8	70.0	255	2,222	29.7	43.7	0.680
4	122	4.00	11.9	58.0	290	2,000	27.1	38.3	0.708
5	155	3.64	11.6	62.4	302	2,220	27.2	37.1	0.733
(193)	†	See note A. Fed meat							
6	211	1.86	12.5	142.7	258	4,350	29.5	43.6	0.677
7	230	1.76	13.5	207.7	228	4,540	37.6	48.7	0.772
8	249	1.68	16.0	293.0	195	4,200	42.7	50.3	0.849
9	266	1.35	20.0	405.0	152	4,120	38.0	50.8	0.748
10	292	1.19	22.4	506.0	132	4,000	37.4	50.1	0.747
11	321	1.24	24.4	616.0	122	3,970	43.5	56.0	0.776
(357)	†	See note B. Xylose given <i>per os</i>							
12	394	1.57	25.4	528.0	158	4,700	45.4	64.9	0.700
13	424	1.93	25.3	473.0	194	4,760	50.1	65.8	0.761
14	461	2.06	25.2	441.0	200	4,880	50.1	69.8	0.718
15	493	2.03	25.1	483.0	180	4,770	54.4	74.8	0.727
Experiment 110. January 4									
1	30	1.84	18.4	229	210	3,570	31.8	43.5	0.731
2	59	2.93	18.4	148	234	2,748	32.4	47.8	0.678
3	90	3.48	18.4	128	258	2,725	33.6	51.0	0.659
(339)	†	See note A. Fed meat							

TABLE 1—Continued

PERIOD	TOTAL CON-CURRENT TIME	URINE VOLUME PER MINUTE, v	UREA		XYLOSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Urea	Xylose	

Experiment 110. January 4—Concluded									
		cc.	mgm. per per cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
4	372	2.09	26.7	512.	203	5,240	55.7	75.0	0.743
5	402	2.03	27.0	510.	201	5,280	53.2	73.8	0.722
6	443	2.22	27.6	513.	200	4,870	57.3	75.0	0.764

Experiment 111. January 6									
		cc.	mgm. per per cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	28	4.14	36.4	428	184	2,580	67.6	80.5	0.840
2	59	4.29	33.5	289	221	2,688	51.4	72.4	0.710
3	92	4.88	30.6	255	259	2,465	56.4	64.5	0.874
	(344)	†	See note A. Fed meat						
4	376	2.38	53.2	1,230	173	5,120	76.4	97.8	0.781
5	407	2.55	56.0	1,157	168	5,000	73.2	105.5	0.684
6	440	2.57	58.9	1,326	162	4,760	80.3	104.8	0.766

Experiment 112. January 8									
		cc.	mgm. per per cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	32	2.45	54.1	1,087	182	4,480	68.5	83.8	0.818
	(299)	†	See note A. Fed meat						
2	331	2.31	63.5	1,600	164	5,265	80.8	103.0	0.784
3	360	2.89	66.7	1,385	155	4,875	83.3	126.2	0.660
4	395	2.95	70.0	1,470	146	4,360	86.1	122.4	0.704
5	422	2.70	75.0	1,765	126	4,540	88.0	135.0	0.652
6	454	2.00	80.7	2,375	105	4,290	81.8	113.5	0.721

Experiment 113. January 11									
		cc.	mgm. per per cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	29	1.02	81.4	3,950	105	7,780	68.8	105.0	0.656
2	63	1.06	79.7	3,195	113	7,740	59.0	100.8	0.584
3	92	1.02	78.1	3,190	121	7,780	57.8	91.1	0.634
	(162)	†	See note A. Xylose given per os						
4	190	2.92	66.7	1,075	259	5,480	65.3	85.8	0.752
5	213	2.78	66.0	1,068	245	5,350	62.5	84.3	0.742
6	235	2.96	65.2	1,094	231	5,160	68.9	91.8	0.750

Experiment 144. April 18									
		cc.	mgm. per per cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	31	1.355	14.5	280.5	140	4,180	36.4	56.2	0.648
2	64	1.635	14.2	239.5	148	3,850	38.3	59.1	0.648
3	94	1.600	13.9	239.5	146	3,850	38.3	58.6	0.654
4	151	1.260	13.5	271.2	122	3,500	35.2	50.2	0.702
5	220	0.696	12.8	425.5	62*	2,990	32.1	46.6	0.689

TABLE 1—*Concluded*

PERIOD	TOTAL CON-CURRENT TIME	URINE VOLUME PER MINUTE, V	UREA		XYLOSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA CM. XYLOSE
			Plasma	Urine	Plasma	Urine	Urea	Xylose	

Experiment 144. April 18—*Concluded*

		cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
6	262	0.487	12.3	559.0	30*	2,428	30.7	54.7	0.561
7	280	0.333	12.0	660.0	22*	2,169	23.1	45.6	0.506

Experiment 145. Dog 43

1	30	1.66	16.8	241	172	3,520	33.1	47.2	0.702
2	60	2.00	16.6	211	191	3,595	35.3	52.3	0.675
3	92	1.72	16.4	211	200	3,910	30.7	46.8	0.658
	(186)	See note A. Xylose and urea <i>per os</i> and urea subcutaneously							
4	225	3.25	189.3	1,791	177	2,292	42.7	58.5	0.730
5	285	2.62	209.2	2,122	165	2,325	36.9	51.2	0.720
6	345	1.58	188.0	3,330	82	2,100	38.9	56.2	0.692

Experiment 151. Dog 30

1	22	6.90	5.7	32.8	109	979	46.2	72.0	0.642
2	44	5.10	5.7	44.2	109	1,325	46.0	72.1	0.638
3	67	3.52	5.8	63.9	111	1,830	45.1	67.4	0.669
		See note A. Fed cracker-meal, butter and sugar							
4	91	3.37	6.1	75.3	110	1,910	48.4	68.0	0.712
5	115	2.83	6.4	92.0	98	2,220	47.3	74.6	0.632
	(314)	† See note B. Xylose given <i>per os</i>							
6	329	5.47	5.4	43.2	89	1,035	50.9	73.0	0.698
7	348	5.36	5.2	43.6	93	1,110	52.2	74.4	0.702
8	359	3.73	5.2	67.0	99	1,800	55.9	78.8	0.710

* Determined by adding 100 mgm. per cent glucose to blood filtrate after yeast extraction. Under these conditions the normal blood non-fermentable blank is about 2 mgm. per cent.

† Urine from wash-out period discarded.

cuss it at this time. Our observations that feeding meat increases the rate of excretion of urea relative to the blood urea confirms Addis and Drury (1923) who observed a similar result in man after feeding milk, a mixed meal, etc.

DISCUSSION. The most interesting aspect of these experiments relates to the effect of diet upon the glomerular clearance.

That the glomerular clearance in animals other than the dog is neither constant nor maximal is established by several lines of evidence.

In his Harvey Lecture of 1920-21, Richards (1922) described experiments made with Plant (1917), showing that in the perfused mammalian kidney

small doses of adrenalin have a vasoconstrictor effect (raising the perfusion pressure) though paradoxically causing the kidney to swell. This he explained by the assumption that adrenalin constricts the efferent arterioles of the glomeruli and causes swelling and increased pressure within the glomerular capsule. This increased pressure leads to increased filtration and increased urine formation in spite of the reduced blood flow through the kidney as a whole. Richards also described experiments made with Schmidt on the transilluminated frog's kidney in which alternation of glomerular activity was observed in the living animal, some glomeruli showing rapid blood flow and others a sluggish flow as though the efferent arteriole were greatly constricted. These observations were extended in subsequent communications (Richards and Plant, 1922a, b; Richards and Schmidt, 1924). The number of active glomeruli may be increased by vaso-dilator agencies, etc. (section of sympathetics, injection of NaCl solution, glucose, urea, caffeine and pituitrin in small doses) and decreased by vaso-constrictor agencies (afferent nerve stimulation, hemorrhage, injection of adrenalin or pituitrin in large amounts). Further evidence of the constriction of the efferent arterioles in the frog glomerulus as induced by barium was presented by Mendenhall, Taylor and Richards (1924), and as induced by adrenalin, by Richards, Barnwell and Bradley (1927). White (1930) has more recently observed changes in the blood flow of the transilluminated kidney of *Necturus*, when the glomerular capillaries became congested and dilated. The efferent arteriole was observed to be noticeably constricted.

Bieter (1929) has confirmed the intermittent blood flow in the transilluminated frog's kidney as described by Richards and Schmidt, and the rôle of the sympathetics in maintaining this intermittency. Bieter has also called attention to the appearance in some glomeruli in the frog of a short capillary which connects the afferent with the efferent arteriole, thus affording a "shunt" which permits a rapid blood flow through the glomerulus, while circumventing any great filtration into the capsule. This observation is particularly interesting in that it affords an anatomical basis for glomerular inactivity while insuring continued circulation to the tubule through the efferent arteriole.

In mammals the first recorded evidence of intermittent glomerular function, apart from Richards and Plants' experiments with the perfused kidney, were afforded by Khanolkar (1922) who tried to demonstrate this intermittency by injection methods. After trying a variety of injection materials Khanolkar used carmine and hemoglobin; particularly with the latter he found in frozen sections of injected rabbit's kidney numerous glomeruli showing hemoglobin deposits. He concluded that not all the glomeruli are active at one time and that diuresis (saline plus caffeine) increased the proportion of active units. (The use of hemoglobin is open to

TABLE 2
Effect of diet upon glomerular clearance
 (All experiments on dog 36)

PERIOD	TOTAL CON- CURRENT TIME	URINE VOLUME PER MINUTE, V	UREA		SUCROSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA CM. SUCROSE
			Plasma	Urino	Plasma	Urine	Urea	Sucrose	

Experiment 122. February 1									
		cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	30	0.917	75.7	4,340	118.8	10,920	73.0	117.1	0.623
2	58	0.750	75.4	4,740	112.8	12,220	65.5	113.0	0.580
3	91	0.666	72.8	4,990	110.1	13,360	63.4	112.2	0.565
4	119	0.732	71.6	5,030	98.1	12,890	71.4	133.5	0.535
5	151	0.937	67.4	4,110	119.1	12,650	79.3	138.1	0.574
6	181	0.800	64.9	4,635	104.0	12,650	79.3	135.2	0.586
7	205	0.771	64.5	4,545	92.0	11,510	75.5	134.0	0.563
8	230	0.640	63.9	5,090	78.5	10,870	70.8	123.1	0.574

Experiment 125. February 8									
1	32	0.706	7.5	338	171.8	14,000	44.2	80.0	0.552
2	62	0.780	7.2	298	160.1	12,733	44.9	86.1	0.522
3	125	0.627	7.2	365	140.3	13,025	44.2	80.8	0.547
4	157	0.484	7.2	492	97.0	13,390	45.9	92.8	0.495
5	192	0.365	7.2	643	69.3	12,350	45.3	90.4	0.501
6	232	0.247	7.2	864	48.0	11,145	41.1	79.6	0.516

Experiment 126. February 9									
1	40	0.877	8.3	287	254.8	14,500	42.1	69.4	0.606
2	80	0.635	8.3	345	180.2	14,350	36.7	70.3	0.522
3	120	0.378	9.0	530	121.7	15,325	30.9	66.1	0.467
4	160	0.300	9.1	728	74.0	14,000	33.3	78.8	0.423
5	199	0.202	9.2	1,027	44.7	12,000	31.3	75.3	0.416

Experiment 137. March 29									
1	60	0.463	14.4	744	135.4	9,575	33.2	45.5	0.746
2	114	0.363	13.8	752	101.9	9,795	27.5	48.5	0.567
3	174	0.325	13.4	892	68.4	8,010	30.0	52.8	0.566
4	237	0.232	13.3	1,141	42.9	5,130	27.6	38.5	0.717

the objection that it produces profound changes in kidney volume, and probably disturbances in glomerular activity—a danger which attends all injection methods. Cf. Reid, 1929 and Mason and Mann, 1931.) Hayman and Starr (1925), from injection experiments with Janus green, concluded that the number of open glomeruli in the rabbit's kidney varies widely under spontaneous and experimental conditions. The proportion

of active glomeruli is increased by caffeine and saline and decreased by adrenalin and CO₂ inhalation.

Sheehan (1931) has observed that after injection of dyes into rabbits some tubules may be stained while others are not, which fact indicates intermittency of activity.

Bensley (1929) has examined the efferent arterioles in mammals and describes a network of cells (pericytes) investing them which resemble Rouget cells somewhat in the smaller animals, but which approach true smooth muscle cells in structure in the case of man. He considers these pericytes to be a mechanism admirably adapted for the intrinsic control of glomerular activity and pressure.

Winton (1931a, b), working with the isolated dog kidney, has reached conclusions in line with the observations which Richards has made upon the frog; the quantity of glomerular filtrate can be increased by dilatation of the afferent arterioles and constriction of the efferent arterioles (adrenalin in small doses, etc.) while constriction of the afferent arterioles (larger doses of adrenalin) reduces the quantity of glomerular filtrate. According to Winton, caffeine increases the glomerular pressure and blood flow by dilatation of the afferent arteriole; low concentrations of adrenalin have the same effect on the glomerular pressure but reduce the blood flow by constricting the efferent arteriole; higher concentrations of adrenalin reduce glomerular pressure and blood flow by constricting the afferent arteriole. Pituitary extracts, according to Winton, reduce the urine flow by increasing the rate of reabsorption of water and not by a vasomotor action.

The above observations supplement our present findings that the glomerular activity varies over a wide range in the normal, intact, unanesthetized dog. There is nothing in our data on the effect of diet to indicate how these changes in glomerular activity are brought about in the kidney; i.e., whether by changes in effective glomerular filtration pressure or total filtering surface. The former might result from changes in efferent or afferent arterial tone, total blood flow through kidney or systemic arterial pressure, and the latter from changes in the total number of active glomerular capillaries, whether mediated by "shunts" or otherwise. (We know of no histological evidence which definitely precludes the existence of capillary shunts between the afferent and efferent arterioles in the mammal similar to those observed by Bieter in the frog.) Nor is there evidence here to enable us to decide whether the changes in glomerular activity are effected through some humoral factor associated with the high protein metabolism characteristic of a meat diet, or whether these changes are effected by way of the sympathetic nervous system. In addition, it is recognized that there may be changes in the protein content of the plasma brought about by diet, but these would be expected to act in the reverse

direction since the plasma protein should rise and tend to retard glomerular filtration on a high protein diet.

But it is possible to conclude from these data that there are important changes in the functional activity of the glomeruli associated with metabolism, and that these changes in functional activity tend to be cumulative so that the net result of a week's maintenance on a particular diet may be much more marked than the results of a single meal, even when the functional activity of the kidneys is observed in the standard, post-absorptive condition.

It would appear that a similar phenomenon occurs in man if we may make an approximation from the data on Deuel, Sandiford, Sandiford and Boothby (1928). During the period when Deuel was living on a low protein diet the blood ureas remained consistently at 14 mgm. per cent. Doctor Boothby has kindly furnished us with the urine volumes which enable us to calculate Deuel's urea clearance from the 24 hour data. The standard clearance $\left(\frac{U\sqrt{V}}{B}\right)$ was 53.4 at a urine flow of 0.475 cc. per minute on the first day on the low protein diet, and fell to 17.5 on the third day (urine flow, 1.21 cc. per minute) and to 9.6 on the 32nd day (urine flow, 0.347 cc. per minute). If the urea clearance parallels the glomerular clearance in man as closely as it has in the experiments reported here on the dog, these calculations would indicate that Deuel's glomerular filtrate was very significantly reduced by subsistence on a low protein diet.

SUMMARY

Observations on a single dog fed alternately on a cracker meal diet and a meat diet show that the glomerular clearance (xylose or sucrose clearance) can be increased nearly $3\frac{1}{2}$ -fold by feeding meat, i.e., from 40 to 135 cc. per square meter of body surface per minute.

The glomerular clearance can be increased two-fold in the course of 4 or 5 hours after a single meal of raw beef but the maximal effect is obtained after feeding meat when meat is also used as a maintenance diet. The glomerular clearance tends to persist at an elevated level for some hours or days after meat feeding, so that the effect of a meat diet is cumulative.

Evidence is presented in favor of the view that this elevation in the glomerular clearance is largely due to increased glomerular filtration, rather than to hypertrophy of the kidneys.

While on a meat diet the glomerular clearance tends to be erratic and may fall abruptly to intermediate levels, which again argues against this elevation being due to hypertrophy of renal tissue.

The urea clearance is invariably less than the glomerular clearance. There is no change in the urea:glomerular clearance ratio with changes in urine flow above 1.0 cc. per minute, and with changes in plasma urea from

16.4 to 209.2 mgm. per cent, or with changes in urine urea from 211 to 3330 mgm. per cent. Beyond noting these facts, no comment is made on this point.

Protocols Referring to Data in Tables Showing Effect of Diet Upon Glomerular Clearance. Dog 36: Weight throughout = 15 kgm., surface area = 0.72 sq. m. Female collie. *Experiment 106.* . . . December 23. Cracker meal diet 22 days, water *ad lib.* Given 600 cc. water by stomach at 8:30 a.m. Forty-five grams xylose in 600 cc. water by stomach at 9:40 a.m. Fifteen grams xylose in 225 cc. water by stomach at 10:15 a.m. Period 1 began at 10:50 a.m. Blood drawn at 3, 74, 156, 205, 272, 320 and 350 minutes, and urea and xylose interpolated for middle of each urine period. Note A: 30 grams xylose in 225 cc. water by stomach at end of period. Note B: 45 grams xylose in 90 cc. water at end of period 9. At beginning of period 9 dog saw meat being prepared for other dogs.

Experiment 107. Dog 36. December 30. Cracker meal diet 29 days. Water *ad lib.* Given 45 grams xylose in 450 cc. water by stomach at 7:56 a.m., 15 grams xylose in 150 cc. water by stomach at 8:30 a.m. Period 1 began at 9:00 a.m. Blood drawn at 15, 92, 157, 212, 268, 325, 375, 426 and 495 minutes, and urea and xylose interpolated for middle of each urine period. Note A: at end of period 5 dog ate 900 grams raw beef in which were mixed 45 grams xylose. Note B: 30 grams xylose in 300 cc. water by stomach at end of period 11.

Experiment 110. Dog 36. January 4. Meat diet since December 30. There is a possibility dog did not eat meat the day before this experiment. Given 45 grams xylose in 600 cc. water by stomach at 8:15 a.m., 15 grams xylose in 225 cc. water by stomach at 8:45 a.m. Period 1 began at 9:15 a.m. Blood drawn at 15, 75, 355 and 421 minutes and urea and xylose interpolated. Note A: Dog ate 900 grams of raw beef at end of period 3. Forty-five grams xylose in 600 cc. water by stomach 84 minutes before start of period 4. Hematocrit blood 1 = 37.2 per cent; blood 4 = 36.5 per cent. Total plasma nitrogen blood 4 = 1071 mgm. per cent.

Experiment 111. Dog 36. January 6. Meat diet since December 30. Water *ad lib.* Given 45 grams xylose in 600 cc. water by stomach at 8:10 a.m., 15 grams xylose in 225 cc. water by stomach at 8:40 a.m. Period 1 began at 9:17 a.m. Blood drawn at 13, 74, 359 and 419 minutes, and urea and xylose interpolated for middle of each urine period. Note A: Dog ate 900 grams raw beef at end of period 3. Forty-five grams xylose in 600 cc. water by stomach 75 minutes before beginning of period 4. Hematocrit, blood 4 = 30.0 per cent. Total plasma nitrogen blood 4 = 923 mgm. per cent.

Experiment 112. Dog 36. January 8. Meat diet since December 30. Dog ate 900 grams raw beef twice daily January 6 and 7. Forty-five grams xylose in 600 cc. water by stomach at 8:15 a.m. Period 1 began at 9:15 a.m. Note A: Dog ate 900 grams raw beef at end of period 1, 45 grams xylose in 600 cc. water by stomach 59 minutes before beginning of period 2. Blood drawn at 15, 315, 375 and 435 minutes and urea and xylose interpolated.

Experiment 113. Dog 36. January 11. Meat diet since December 30. No water allowed since 9:00 a.m. January 9. Dog ate 900 grams raw beef January 8, 900 grams January 9, 900 grams at 11:00 a.m. and 900 grams at 5 p.m. January 10. On January 11, 30 grams xylose in 400 cc. water injected subcutaneously at 8:15 a.m. Seven and one-half grams xylose in 100 cc. water injected subcutaneously at 8:45 a.m. Period 1 began at 9:00 a.m. Note A: At end of period 3, 30 grams xylose in 600 cc. water given by stomach. Thirty-seven minutes before beginning of period 4, 15 grams xylose in 225 cc. water given by stomach. Blood drawn at 15, 75, 177 and 224 minutes

and urea and xylose interpolated. Hematocrit blood 1 = 37.0 per cent; blood 2 = 39.0 per cent. Total plasma nitrogen, blood 1 = 984 mgm. per cent.

Experiment 122. Dog 36. February 1. Mixed diet since January 11. Dog ate 900 grams raw beef January 30 and 31. Water removed from cage 2 p.m. January 31. On February 1 fed 900 grams raw beef at 7 a.m. Drank 360 cc. water at 8:15 a.m. Ten and one-half grams sucrose in 150 cc. water injected subcutaneously at 8:25 a.m. and 4.5 grams sucrose in 50 cc. water injected subcutaneously at 9:00 a.m. Period 1 began at 9:31 a.m. All blood samples drawn at middle of urine periods. At 93 minutes gave 600 cc. water by stomach, and at 94 minutes 7.5 grams sucrose in 100 cc. water subcutaneously. Hematocrit, blood 8 = 40.2 per cent.

Experiment 125. Dog 36. February 8. Dog fasted February 2 to 6. Cracker meal diet February 6 to 7. No water since 5:00 p.m. February 6. On February 8, 19.5 grams sucrose in 180 cc. water injected subcutaneously. Period 1 began at 9:13 a.m. All blood samples drawn at middle of urine periods. Hematocrit, blood 7 = 35 per cent.

Experiment 126. Dog 36. February 9. Experiment 125 continued without water. Nineteen and one-half grams sucrose in 200 cc. water subcutaneously at 8:15 a.m. Period 1 began at 9:00 a.m. All blood samples drawn at middle of urine periods. Hematocrit, blood 7 = 30.5 per cent.

Experiment 127. Dog 36. March 29. Mixed diet February 10-March 15. Cracker meal diet March 15-28. No water since 9:00 a.m. March 26. On March 29, 15 grams sucrose in 100 cc. water injected subcutaneously. Period 1 began at 10:03. All blood samples drawn at middle of urine periods.

Experiment 144. Dog 36. April 18. Cracker meal diet since March 15. No water since April 16. On April 18, 22.5 grams xylose in 90 cc. water by stomach at 8:15 a.m. and 7.5 grams xylose in 45 cc. water by stomach at 8:45 a.m. Period 1 began at 9:59 a.m. All blood samples drawn at middle of urine periods.

Experiment 145. Dog 43. Weight 19 kgm., S. A. 0.92 sq. m. April 20. Cracker meal diet since March 15. No water since 5:00 p.m., April 19. Twenty-eight and one-half grams xylose in 190 cc. water by stomach at 9:00 a.m., 9.5 grams xylose in 57 cc. water by stomach at 9:30 a.m. Period 1 began at 10:30 a.m. Note A: At end of period 3, 14.3 grams urea and 14.3 grams xylose in 76 cc. water by stomach and 14.3 grams urea in 100 cc. water subcutaneously. All blood samples drawn at middle of urine periods.

Experiment 151. Dog 30. Weight 20 kgm., S. A. 0.86 sq. m. May 11. Cracker meal diet since May 6. Thirty grams xylose in 800 cc. water by stomach at 9:30 a.m., 10 grams xylose in 400 cc. water by stomach at 10:05 a.m. Period 1 began at 11:04 a.m. Note A: At end of period 3 dog ate 100 grams cracker meal, 30 grams sucrose and 30 grams butter. Note B: 30 grams xylose in 800 cc. water by stomach at 205 minutes. Ten grams xylose in 400 cc. water by stomach at 235 minutes.

BIBLIOGRAPHY

- ADDIS, T. AND D. R. DRURY. 1923. Journ. Biol. Chem., *lv*, 629.
 AUSTIN, J. H., E. STILLMAN AND D. D. VAN SLYKE. 1921. Journ. Biol. Chem., *xlvi*, 91.
 BENSLEY, R. D. 1929. Amer. Journ. Anat., *xliv*, 141.
 BIETER, R. N. 1929. This Journal, *xei*, 436.
 DEUEL, H. J., I. SANDIFORD, K. SANDIFORD AND W. M. BOOTHBY. 1928. Journ. Biol. Chem., *lxxxvi*, 391.
 HAYMAN, J. M., JR. AND I. STARR. 1925. Journ. Exp. Med., *xlii*, 641.

- JOLLIFFE, N. AND H. W. SMITH. 1931a. *This Journal*, xcviii, 572.
1931b. *This Journal*, xcix, 101.
- JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH. 1932. *This Journal*, c, 301.
- KHANOLKAR, V. R. 1922. *Journ. Path. and Bact.*, xxv, 414.
- MAC KAY, E. M. 1932. *This Journal*, c, 402.
- MAC KAY, E. M. AND L. L. MAC KAY. 1931. *Journ. Nutri.*, iii, 375.
- MAC KAY, E. M., L. I. MAC KAY AND T. ADDIS. 1928. *This Journal*, lxxxvi, 459.
- MASON, J. B. AND F. C. MANN. 1931. *This Journal*, xcviii, 181.
- MENDENHALL, W. L., E. M. TAYLOR AND A. N. RICHARDS. 1924. *This Journal*, lxxi, 174.
- REHBERG, P. B. 1926. *Biochem. Journ.*, xx, 461.
- REID, W. L. 1929. *This Journal*, xc, 168.
- RICHARDS, A. N. 1920-21. *Harvey Lectures*, xvi, 163.
1922. *Amer. Journ. Med. Sci.*, clxiii, 1.
- RICHARDS, A. N., J. B. BARNWELL AND R. C. BRADLEY. 1927. *This Journal*, lxxix, 410.
- RICHARDS, A. N. AND O. H. PLANT. 1917. *This Journal*, xlii, 592.
1922a. *This Journal*, lix, 184.
1922b. *This Journal*, lix, 191.
- RICHARDS, A. N. AND C. F. SCHMIDT. 1924. *This Journal*, lxxi, 178.
- SHEEHAN, H. L. 1931. *Journ. Physiol.*, lxxii, 201.
- WHITE, H. L. 1930. *Proc. Soc. Exp. Biol. Med.*, xxvii, 613.
- WINTON, F. R. 1931a. *Journ. Physiol.*, lxxii, 361. lxxiii, 151.

THE EXCRETION OF URINE IN THE DOG

V. THE EFFECTS OF XYLOSE AND SUCROSE UPON THE GLOMERULAR AND UREA CLEARANCES

NORMAN JOLLIFFE, JAMES A. SHANNON AND HOMER W. SMITH

*From the Department of Physiology, University and Bellevue Hospital Medical College,
New York City*

Received for publication May 28, 1932

In view of the fact that the glomerular clearance is profoundly modified by diet and other physiologically active agents (cf. Shannon, Jolliffe and Smith, 1932) it is pertinent to inquire if xylose or sucrose themselves exert any action upon renal activity. It is obvious that this question can be answered only by reference to the rate of excretion of some substance other than xylose or sucrose, and at the present time urea is the only substance suitable for this purpose. We have shown that at rates of urine flow above 1 cc. per minute the ratio of the urea to the glomerular clearance was essentially constant for one dog (0.65-0.75), although the glomerular clearance was increased three and a half fold by feeding meat. In view of this fact we feel that if it could be shown that neither xylose nor sucrose exerted any significant action upon the urea clearance, this fact might be advanced as evidence that these sugars did not *per se* influence the glomerular clearance when administered to the normal dog. The point, we think, is singularly important because of the great variations in the glomerular clearance which we have observed in dogs to which these sugars have been administered for the purpose of measuring the glomerular filtrate.

As a preface to the investigation of this question it is necessary to examine certain conditions of experiments of this nature. A serious difficulty, and one to which reference is rarely made in studies of renal activity, is the observational error introduced by the dead space of the tubules, ureters and bladder; this error becomes particularly significant when the rate of urine flow is changing rapidly; at such times it may lead to entirely misleading results in the calculation of the glomerular clearance (or the clearance of urea or any other substance, for that matter). This dead space error can, of course, be avoided to a great extent by diuresis induced by the preliminary administration of large quantities of water (as in

the standard conditions of Addis (1922) and Taylor, Drury and Addis (1923)).¹

But a second difficulty is the marked sensitivity of the glomeruli in the normal animal. A phenomenon of special interest here is that the administration of water (or salt solution) by stomach may lead to an increase in the glomerular clearance under conditions where there is no significant change in the rate of urine flow (and therefore no dead-space error), or when an appropriate correction for the dead-space is applied.

The first experiment which we wish to describe illustrates both of the above points well.

Experiment 103 (table 1) was performed upon a dog subsisting on a cracker meal diet and which has been used frequently in experiments of this kind and was therefore thoroughly accustomed to the routine. The urea clearance at natural urine flows (periods 1 and 2), which lay below the augmentation limit, was first determined early in the morning. Water was then given by stomach; as diuresis developed a urine containing concentrated urea from period 3 was swept out of the kidney by the less concentrated urine of period 4 which was now flowing at a greatly increased rate. The result of the dead-space error here is to produce an apparent urea clearance of 114, as compared with the probable clearance of 50 (the rate of urine flow was increasing from 0.094 to 0.856 cc. per minute). A similar effect is evident in period 5 in which the apparent clearance is 71.0, since the rate of urine flow is still increasing (0.856 to 4.66 cc. per minute). The effect of the dead-space can be illustrated by a simple calculation on period 5: during this period 200 mgm. of urea were excreted; if we assume that the dead-space is 8 cc. and deduct 87.5 mgm. (0.08×1094) for urea left from period 4 and add 6.8 mgm. (0.08×85.5) for urea properly belonging to period 6, we arrive at a true excretion for period 5 of 119.3 mgm. or a true urea clearance of 48.8 instead of 71.0. Thus the difference in the urea clearance in periods 5 and 6 appears to be largely an error which is due to a rapidly increasing rate of urine flow.

Dead-space error is not the only factor here, however, as is shown by the next two periods. After another dose of water by stomach the urea clearance increased from 49.2 (period 6) to 60.8 (period 7) although there was no change in urine flow in period 7 as compared with period 6. Apparently the administration of water in this case really increased the urea clearance. This phenomenon occurs again after the fourth dose of water (periods 8 and 9), yet when a correction is applied to period 9, on the basis

¹ In our previous experiments on diet, etc., dead-space error was avoided by the preliminary administration of water as shown in our protocols, and in our experiments comparing the excretion of xylose, glucose, sucrose and raffinose (Jolliffe, Shannon and Smith, 1932), apart from the water administered beforehand to induce diuresis, no water was administered by stomach during the experiments.

of an 8 cc. dead-space, this correction does not change the urea clearance from the observed figure. It may be noted that the urea clearance invariably falls during the second thirty-minute period after the administration of water, a fact which supports the idea that the clearance has previously been increased by the administration of water.

It is evident, however, that by the end of period 10 the urea clearance is fairly constant. At this time the same quantity of water was administered as before, but with a suitable quantity of xylose dissolved in it. The urea clearance increased very slightly, but no more than between periods 6 and 7 or between 8 and 9. At the end of period 12, water and xylose were again administered and produced the customary slight rise in the urea clearance.

By the end of period 14, it was possible to observe the glomerular clearance as measured by the excretion of xylose.

A large quantity of water with xylose now produced the usual slight rise in urea clearance (if corrected for an 8 cc. dead-space the urea clearance for period 15 would still be 55.0, as compared with 51.8 in the previous period); and what is more noteworthy, a corresponding rise in the glomerular clearance. Numerous experiments which will not be reported in detail here indicate that the increase in the urea clearance which results from the administration of water *per os* is due to a corresponding increase in the glomerular clearance.

The effect of the enteric administration of water (in the form of xylose solutions) on the glomerular clearance is also illustrated in experiments previously published by us (Shannon, Jolliffe and Smith, 1932); experiment 106, periods 6-7, 42.5 to 52.4; experiment 107, periods 11-12, 56.0 to 64.9; experiment 145, periods 3-4, 46.8 to 58.5. None of these instances can be explained on the basis of dead-space alone, and it appears from experiment 103 that the phenomenon is not due to the xylose since the administration of xylose solutions exerts no greater effect than does the administration of water only.

It appears from these observations that this water-effect represents an increase in the glomerular clearance which is followed more or less passively by the urea clearance. We have described the phenomenon here because of the necessity of considering it in relation to the subject matter of this paper, although we have no explanation to offer for it at this time.

The next experiment (exp. 89, table 1) concerns the effect of xylose when administered subcutaneously on the urea clearance. In this experiment the urea clearance was not observed immediately after the xylose injection; at the end of an hour and a half, however, when the glomerular clearance could be measured by the excretion of xylose the urea clearance was close to what it had been in the last of the control periods—the injection of xylose had had no effect upon it.

Experiment 120 (table 2) concerns the effect of sucrose injected subcutaneously on the glomerular clearance (xylose) and the urea clearance, as observed in a dog on a mixed diet. In this case the xylose was adminis-

TABLE 1
The effects of the administration of xylose on the urca clearance in dogs

PERIOD	TOTAL CON- CURRENT TIME	URINE VOLUME	UREA		XYLOSE		CM. = $\frac{UV}{P}$ / S.A.		CM. UREA
			Plasma	Urine	Plasma	Urine	Urea	Xylose	CM. XYLOSE
Expt. 103. Dog 36									
	minutes	cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.			
1	60	0.150	9.9	1,479			31.1		
2	95	0.072	10.7	2,370			22.1		
40 cc. water per kgm. by stomach									
3	127	0.094	11.4	2,326			26.6		
4	158	0.856	11.4	1,094			114.1		
15 cc. water per kgm. by stomach									
5	192	4.66	11.5	126.2			71.0		
6	220	4.89	11.5	85.5			49.2		
15 cc. water per kgm. by stomach									
7	244	4.95	11.4	100.7			60.8		
8	283	4.69	11.2	94.4			54.9		
15 cc. water per kgm. by stomach									
9	310	5.48	10.9	84.0			58.6		
10	341	4.39	10.7	93.4			53.2		
15 cc. water and 2.5 grams xylose per kgm. by stomach									
11	372	1.45	10.5	295.0			56.6		
12	404	3.19	10.4	118.2			50.4		
15 cc. water and 0.5 grams xylose per kgm. by stomach									
13	437	2.87	10.3	143.9	135.0	2,885	55.6	85.2	0.652
14	468	2.80	10.1	134.4	151.5	2,775	51.8	71.2	0.628
40 cc. water and 0.5 gram xylose per kgm. by stomach									
15	500	4.19	9.8	96.8	157.7	2,082	57.5	76.8	0.748
16	532	5.78	9.5	64.4	159.6	1,500	54.4	75.4	0.721
Expt. 89. Dog 30									
1	32	2.69	42.8	106			77.4		
2	62	5.49	38.3	445			74.2		
(122) See note A. Xylose subcutaneously									
3	152	1.20	34.8	1,730	82	5,870	69.4	100.0	0.694
4	182	1.03	31.2	1,892	83	7,060	72.6	102.0	0.711

tered by stomach beforehand with sufficient water to produce a prolonged diuresis, and the glomerular and urea clearances were observed without interruption after the injection of sucrose. Neither the urea nor the

glomerular clearance was affected by the sucrose beyond the small variations which we have observed to occur spontaneously. Experiments 89 and 120 show that xylose and sucrose do not affect the glomerular or the urea clearance when these are elevated by a meat diet, while experiment

TABLE 2

The effects of administering xylose and sucrose on the urea and glomerular clearances in dogs

PERIOD	TOTAL CONCURRENT TIME	URINE VOLUME	UREA		XYLOSE		SUCROSE		CM. $\frac{UV}{P}$ / S.A.			CM. UREA CM. XYLOSE	CM. XYLOSE CM. SUCROSE
			Plasma	Urine	Plasma	Urine	Plasma	Urine	Urea	Xylose	Sucrose		
Expt. 120. Dog 20													
	min-utes	cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.					
1	83	2.75	63.7	1,682	82	2,732			95.5	120.5		0.792	
2	99	4.12	62.3	989	86	1,859			86.0	117.2		0.734	
3	120	4.19	61.3	1,047	106	2,200			94.1	114.5		0.821	
See note A: Sucrose and more xylose subcutaneously													
4	179	5.29	57.5	746					90.2				
5	201	3.63	53.2	1,069	136	3,410	111	2,885	96.0	119.7	124.1	0.802	0.965
6	223	3.91	51.5	923	136	2,986	106	2,288	92.2	112.9	111.0	0.817	1.018
7	240	4.47	50.2	805	136	2,552	104	1,926	94.3	110.4	109.0	0.854	1.012
Expt. 146. Dog 43													
1	41	1.12	20.4	471					28.1				
2	64	0.91	20.4	579					28.1				
3	84	0.70	19.7	720					27.8				
See note A: xylose by stomach													
4	127	0.71	18.7	732					30.2				
5	148	1.10	18.0	405	140	4,285			26.9	36.5		0.737	
6	166	1.56	17.9	337	174	4,385			31.9	42.7		0.747	
7	185	1.48	17.6	292	189	4,235			26.7	36.1		0.740	
See note B: sucrose subcutaneously													
8	214	1.66	17.3	260					27.1				
9	245	2.19	17.0	181	202	3,625			25.3	42.7			
10	265	2.05	17.3	188	212	3,400	208	3,270	24.4	35.8	35.0	0.681	1.023
11	304	2.20	17.6	195	219	3,625	246	4,080	26.5	39.6	39.7	0.669	0.998
12	324	2.10	17.9	229	178	3,355	256	4,850	29.2	43.0	43.3	0.679	0.994

103 shows the absence of any action by xylose at the reduced levels of glomerular activity which are characteristic of a cracker meal diet.

It seemed that the most exacting experiment for demonstrating the physiological indifference of xylose and sucrose would be one in which the

conditions were such as to give a reduced, steady glomerular clearance, and one in which the effect of water administration was eliminated as far as possible. To obtain these conditions, a dog was used which had been kept upon a cracker meal diet for 11 days and in which the clearance was known to be down to the typical, basal level. (Cf. expt. 146, table 2.) Ample water was administered to produce a copious diuresis; the water was given four hours beforehand so that the experiment could be conducted entirely in the post-diuretic period.

After three control periods, during which the urea clearance was observed to average 28.0, xylose in concentrated solution was administered by stomach. In the next 43 minute period the urea clearance rose to 30.2 (a negligible increase) while the average of four periods extending over an hour and a half was 28.9. Then sucrose and a small quantity of xylose were injected subcutaneously; the urea clearance remained essentially unchanged in the next half-hour period, and for the next two hours and a quarter averaged 26.5.

The glomerular clearance, as measured by the xylose excretion, in periods 5, 6 and 7 averaged 38.4; after sucrose, in periods 9, 10, 11 and 12, it averaged 40.3. The xylose:urea ratio changed from 0.74 before sucrose to 0.68 after sucrose, but since a ± 3.0 per cent error in the urea and xylose clearances at 30 and 40, respectively, produces a change in the ratio of these clearances of 0.09, the observed change of 0.06 cannot be considered to be particularly significant.

Thus, under the conditions of this experiment, the urea clearance remained fairly constant although sufficient xylose was administered by mouth, and sufficient sucrose parenterally, to permit the determination of the rate of glomerular filtration by either sugar, and in spite of the fact that the concentration of total sugar in the urine reached 8 per cent. Furthermore, the sucrose did not significantly affect the glomerular clearance as measured by xylose.²

The above observations were all made at moderate to high rates of urine flow, and therefore with correspondingly low concentrations of xylose and sucrose in the urine (4.4 and 3.6 per cent, respectively), and it cannot be argued from them that these sugars, if present in the urine in very high concentrations, would not modify the normal urea clearance. (Sucrose may be concentrated to 25 per cent in the dog.) We do not believe that this question can be answered by experiments of the type which we have used here, since below the augmentation limit the rate of excretion of urea is intimately related to the rate of urine flow (Austin, Stillman and Van Slyke, 1921).

² The correspondence between the xylose and sucrose clearances in experiments 120 and 146 confirms our previously recorded observations that these sugars are excreted, in simultaneous experiments, in an identically quantitative manner.

Our experiments are sufficient, however, to assure us that in using xylose and sucrose to measure the glomerular clearance we are not administering to the animal substances which modify either the state of activity of the glomeruli, or the urea clearance.

It is also appropriate to refer at this time to the fact that dog 36 (see Shannon, Jolliffe and Smith, 1932) received xylose and sucrose repeatedly over a period of four months without any evidence of impairment in renal function. The most significant criterion of this fact, we think, is the constancy of the urea : glomerular clearance ratio throughout the period. We emphasize this ratio because we feel that tubular injury (whether acute or chronic) will probably first reveal itself by an increased permeability and consequently an increased diffusion of urea back into the renal blood and lymph, as has been suggested by Rehberg (1926) and Holten and Rehberg (1931). The result of this diffusion will of course be to lower the urea : glomerular clearance ratio.

SUMMARY

It is pointed out that the administration of water by stomach may lead to an increase in the glomerular (and therefore the urea) clearance.

It is shown that in properly conducted experiments (and under conditions in which the urea clearance has been shown to parallel the glomerular clearance), the administration of xylose solutions by stomach or by subcutaneous injection does not significantly modify the urea clearance; and that the subcutaneous injection of sucrose solutions does not significantly modify either the xylose clearance or the urea clearance.

It is concluded that xylose and sucrose are physiologically inert, so far as renal function is concerned, if used in the manner described here, and may safely be used to measure the glomerular clearance without danger of perturbing effects upon the glomeruli, or upon the excretion of urea.

Protocols accompanying experiments in tables 1 and 2. Experiment 103. Dog 36. Weight 15 kgm., S. A. 0.72 sq. m. Cracker meal diet, 12 days. Water and xylose given by stomach tube as indicated in table. Blood drawn at 0, 98, 222, 374, 422, 457, 483 and 516 minutes. Plasma urea and xylose concentrations interpolated to middle of each urine period.

Experiment 89. Dog 30. Weight 15 kgm., S. A. 0.86 sq. m. Meat diet, one week. Eighteen grams xylose in 100 cc. water subcutaneously at 62 minutes and 4.5 grams xylose in 20 cc. water subcutaneously at 107 minutes. All blood samples drawn at middle of urine periods.

Experiment 120. Dog 20. Weight 18 kgm., S. A. 0.76 sq. m. Mixed diet 47 days. Fifty-four grams xylose in 720 cc. water by stomach at 8:15 a.m. and 9 grams xylose in 270 cc. water by stomach at 8:45 a.m. Period 1 began at 9:14 a.m. Note A.: 18 grams sucrose in 180 cc. water subcutaneously at 125 minutes and 18 grams xylose in 270 cc. water by stomach at 135 minutes. All blood samples drawn at middle of urine periods.

Experiment 146. Dog 43. Weight 19 kgm., S. A. 0.92 sq. m. Cracker meal diet 16 days. Seven hundred sixty cubic centimeters water by stomach at 8:15 a.m.

Bladder emptied and period 1 began at 12:15 p.m. Note A: 38 grams xylose in 38 cc. water by stomach at 86 minutes. Note B: 38 grams sucrose and 9 grams xylose in 150 cc. water subcutaneously at 189 minutes. All blood samples drawn in middle of collection periods.

BIBLIOGRAPHY

ADDIS, T. 1922. Arch. Int. Med., xxx, 378.

AUSTIN, J. H., E. STILLMAN AND D. D. VAN SLYKE. 1921. Journ. Biol. Chem., xli, 91.

HOLTEN, C. AND REHBERG, P. B. 1931. Acta. Med. Scand., lxxiv, 479.

JOLLIFFE, N., J. A. SHANNON AND H. W. SMITH. 1932. This Journal, c, 301.

REHBERG, P. B. 1926. Biochem. J., xx, 461.

SHANNON, J. A., N. JOLLIFFE AND H. W. SMITH. This Journal, in press.

TAYLOR, F. B., D. R. DRURY AND T. ADDIS. 1923. This Journal, lxv, 55.

THE CHEMICAL CONTROL OF BREATHING, AS SHOWN IN THE ACID BASE BALANCE OF THE BLOOD, UNDER PROGRES- SIVE DECREASE OF OXYGEN¹

YANDELL HENDERSON AND ELLEN M. RADLOFF²

From the Laboratory of Applied Physiology, Sheffield Scientific School, Yale University

Received for publication May 28, 1932

Oxygen deficiency induces an increase of breathing; but through what means or process this influence acts is still obscure. Is this influence, like that of carbon dioxide and that of the blood alkali exerted through the hydrogen ion concentration of the blood? Or does this factor dominate the others and exert a control of a different and more fundamental character?

The problem of the influence of oxygen deficiency is peculiarly difficult; this factor cannot be isolated experimentally. Its part in the chemical control of breathing cannot be studied apart from the influence of the other two factors, carbon dioxide and alkali, and their resultant hydrogen ion concentration. The effects of excess or deficiency of carbon dioxide are easily demonstrated apart from any other factor. The influence of increase or decrease of the alkali in use, or bicarbonate level of the blood, can also be shown independently. But the influence of oxygen deficiency upon respiration manifests itself essentially in the disturbances which it induces in the other factors. It can be brought into view and analyzed only by defining these disturbances.

ANOXEMIA AND ASPHYXIA: ALKALOSIS AND ACIDOSIS. In 1891 Araki (1) made an important and correct observation, which has nevertheless led to a vast deal of error and confusion. He found that in asphyxia, under carbon monoxide, there is a large production of lactic acid. This observation and similar observations under related conditions have been used ever since to explain the increased breathing under moderate decrease of oxygen. It is still a common, but quite erroneous, belief that a slight or moderate decrease of oxygen pressure leads to an increased production of lactic acid in the tissues, and that this acid escaping into the blood neutralizes a

¹ The experimental data upon which this paper is based are contained in a dissertation submitted by the junior author to the faculty of the Graduate School of Yale University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1930.

² Sterling Fellow.

part of its alkalinity, increases its hydrogen ion concentration and thus stimulates respiration. Moderate anoxemia has been confused with asphyxia, and has been conceived in terms of asphyxial acidosis.

In 1914 Barcroft (2) observed that under the lowered barometric and oxygen pressures on the Peak of Teneriffe, the alkali in the blood is diminished. But Ryffel (3) found no increase of lactic acid; and later confirmed this finding on men who spent four hours in a low pressure chamber where the oxygen was reduced to 11.8 per cent of an atmosphere. Marked over breathing occurred, but no increase of lactic acid. In similar experiments Haldane, Kellas and Kennaway (4) also found marked over breathing with no increase of lactic acid, but on the contrary, a decrease of acid and ammonia excretion in the urine: all indications of alkalosis. Henderson (5) independently reached the same conclusion on the basis of experiments on animals.

In a more recent study of the effects of the anoxemia of low barometric pressure Singer (6), using the pneumatic chamber in the laboratory of Professor Hess (7) at Zurich, confirms and extends these observations. He finds that at a barometric pressure of 360 mm., equivalent to 6,000 meters, cyanosis occurs in all persons. There is no decrease of basal metabolism, but a marked diuresis. There is hyperpnea, which Singer believes to be due to the influence of oxygen deficiency directly upon the respiratory center. In contrast to the effects of muscular exertion, the hyperpnea of anoxemia is not due to an acidotic alteration of the blood; on the contrary the hydrogen ion concentration, the titratable acidity and the ammonia of the urine are diminished. Singer finds that in normal men decreased pressure of oxygen—down to 75 mm.—induces no indications of an increased production of acid metabolites.

That oxygen deficiency might induce an acidosis confined to the nervous system is an idea to which Gesell (8) has devoted much experimental work. He argued that such an acidosis of the respiratory center might cause over breathing and thus induce an alkalosis of the blood. Gesell (9), however, in his more recent papers gives weight to the effects of oxygen deficiency upon the respiratory center through some process other than that of acidosis. His conception now appears to be somewhat like that formerly proposed by Loevenhart (10); it accepts oxygen deficiency as an influence independent of the hydrogen ion concentration. Respiration determines pH, instead of pH determining respiration. This conception is the exact opposite of that of Winterstein (11), that the pH is the hormone of respiration, yet, as we shall show later, both in a sense are true.

The opinion that the respiratory center and the nervous system generally might be subjected to an acidotic process, even when other parts of the body were subjected to alkalosis, is one that is on general grounds highly improbable. It is almost entirely lacking in support from valid evidence. The

only evidence for it comes from animals under experimental conditions of virtual asphyxia. In some of Gesell's (12) previous experiments the subjects, dogs, were drugged with heparin, morphine and urethane; their chests were opened and they were throughout the experiment under artificial respiration of uniform volume, but with periods of five to fifteen minutes of diminished oxygen supply. Observations under such conditions may throw light on some of the processes involved in asphyxia; but they are essentially misleading when applied to the problems of normal breathing in unoperated, unanesthetized men and animals.

Conclusive evidence against the theory of an acidosis in the respiratory center under a moderately decreased pressure of oxygen, as a possible cause of alkalosis in the blood, has recently been contributed by Myerson, Loman, Edwards and Dill (13). They have used a technique which enables them to obtain blood directly from the internal jugular vein in man. They find that even when the subjects were breathing air containing only 9 per cent of oxygen, 68 mm.,—the equivalent of an altitude of 22,000 feet—neither the venous blood from the brain nor from a limb contained any more than a normal amount of lactic acid or other acid metabolites.

The evidence from the literature thus demonstrates that mere anoxemia does not induce an increase of lactic acid formation or any other feature of acidosis. There probably is no such condition as anoxemic acidosis. The symptoms mistaken for those of acidosis—over breathing, lowering of the alveolar carbon dioxide and the gradual lowering also of the blood alkali—are not associated with an increase of the hydrogen ion concentration either in the blood or in the nervous system. The pH is raised, not lowered. The erroneous belief on this point still prevalent is based on the application of observations of increased lactic acid and lowered pH under conditions, not of mere anoxemia, but of asphyxia. It is only in asphyxia that an increased production of lactic acid occurs and the pH of the blood is lowered.

The influence of oxygen pressures below normal, but above 60 or 70 mm., is exerted upon the respiratory center, or upon its afferent end organs in the sinus caroticus, in a manner different from that of carbon dioxide and the blood alkali.

EXPERIMENTAL METHODS. In this investigation dogs were used throughout. Altogether twenty-three experiments were made, although all were not complete in all details. Two of the animals were narcotized with "amytal" (iso-amyl-ethyl-barbituric acid in half normal NaOH); but they were found so inert and unresponsive in respect to all physiological readjustments (see fig. 4) that this drug was not used further. Five of the animals received no general anesthetic; the others were given merely enough morphine to quiet them. But all of the animals were carefully protected from pain, anxiety, discomfort or any other condition that could excite

them to increase of respiration. Anoxemia is analgesic, and asphyxia is strongly anesthetic. In all cases novocain was used to anesthetize the skin, before the incisions were made to insert cannulae in the trachea and in the femoral artery for the withdrawal of blood samples. The volume of respiration was not recorded; but the general course of respiration can be inferred with considerable precision from the carbon dioxide content of the arterial blood.

In all cases as soon as the animal was tracheotomized the tracheal cannula was connected with inspiratory and expiratory valves. From one of these valves the animal expired through a cartridge of sodium hydroxide shells into a Douglas bag. Through the other valve the animal inspired again from the Douglas bag. There was thus a closed system containing such a volume of air that in the course of the experimental period the continual breathing of it reduced the oxygen content to a lethal percentage. In all of the experiments here reported, the expired carbon dioxide was absorbed so completely that the air reinspired from the Douglas bag contained not more than a few tenths of one per cent. The air in the bag was analyzed at intervals by means of a Henderson-Orsat analyzer for oxygen and carbon dioxide.

Except in a few cases where the dogs were quite small the blood samples were about 30 cc. They were drawn directly under oil into 3 cc. of an anti-coagulant solution of 3 per cent potassium oxalate and 0.5 per cent sodium fluoride in 0.85 per cent saline. The samples were immediately chilled in a bath of ice water, to obviate the rapid acid change observed by Havard and Kerridge (14).

Determinations of the carbon dioxide content and capacity of the whole blood were made as quickly as possible after the samples were drawn on the constant volume apparatus by the manometric method of Van Slyke and Neill (15). Equilibrations were carried out in a water bath at 37° for twenty to thirty minutes at the carbon dioxide tensions specified in the figures. All the blood samples were fully oxygenated. The method used for estimating lactic acid was that of Friedemann, Cotonio and Shaffer (16); for sugar that of Folin and Wu (17); for inorganic phosphates that of Fiske and Subbarow (18). In determining the hydrogen ion concentration the Dale-Evans (19) modification of the colorimetric method of Levy, Rowntree and Marriott (20) was used. Dialysis tubes were made from a solution of "parlodion" in equal parts of alcohol and ether and dialysis was continued for twenty minutes at 37°C. The standard solution of pH 7.5 used for comparison was electrometrically standardized and was obtained from the La Motte Laboratories. In spite of the criticism of such colorimetric methods, especially by C. J. Johnston (21), the values obtained were considered satisfactory because of their consistency and because differences in pH rather than absolute values were of importance. Bayliss, Kerridge

and Verney (22) from comparison of results by the glass electrode and hydrogen electrode find the dialysis method is accurate to 0.02 pH.

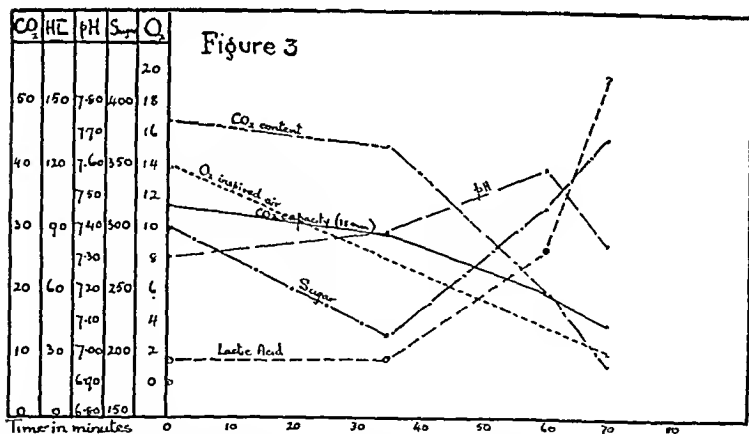
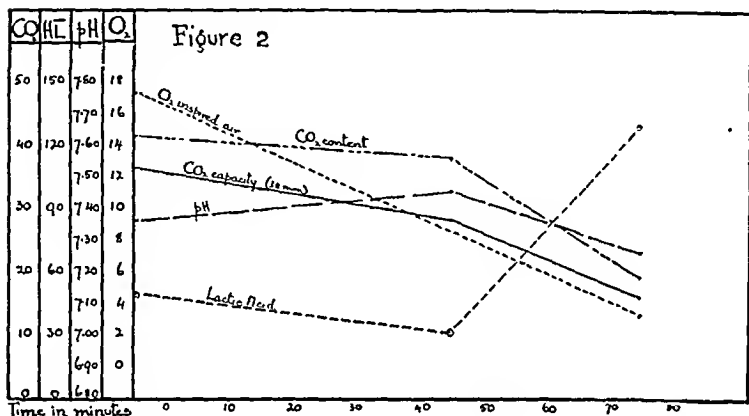
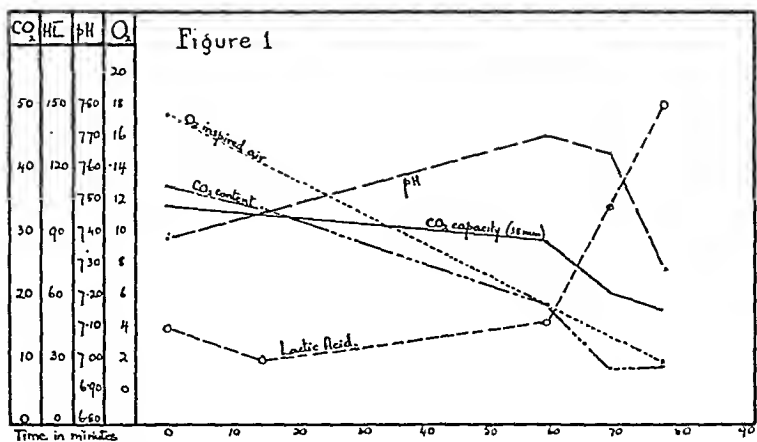
EXPERIMENTAL RESULTS. The principal results of these experiments are illustrated by figures 1, 2 and 3. Each shows the course of events throughout one experiment: the amount of oxygen in the inspired air, and the pH, carbon dioxide content, carbon dioxide capacity and lactic acid content of the blood.

In all these experiments, as is clear from the figures, there are two distinct periods: a first period while the oxygen in the inspired air is falling, but has not yet reached a dangerous degree of depletion; and a second period after a dangerous depletion of oxygen has occurred. During this second period the depletion grows more and more harmful until death results from asphyxia. The critical level of oxygen pressure between these two periods is seen in the figures to be generally at, or a little below, 8 per cent of an atmosphere, 61 mm., of oxygen in the inspired air. This accords with the observations of Jervell (23) and of Mathison (24).

In the first period the significant fact is that as the oxygen falls, the pH does not fall. On the contrary, it rises. In other words, degrees of oxygen depletion above 8 per cent induce, not an acidosis, but an increasing alkalinity in the blood. Furthermore, throughout this first period, the lactic acid content of the blood shows no increase. On the contrary, it remains unchanged from the initial normal level.

The reason for the rise of pH is shown by the relation of the curves for the carbon dioxide content and carbon dioxide capacity; it is especially clear in figure 1. In this experiment the carbon dioxide content of the blood is seen to fall markedly during the first period, while the carbon dioxide capacity falls more slowly. The significance of these facts is that, when respiration begins to respond to a slight deprivation of oxygen, the pulmonary ventilation is increased and the alveolar carbon dioxide is decreased. Consequently the carbon dioxide content of the blood is decreased also and the pH of the blood rises: a rise evidently determined by the decrease in the ratio of $[H_2CO_3]:[BHCO_3]$. The rise of the pH would be much greater except for the fact that the carbon dioxide capacity of the blood, or blood alkali, undergoes a compensatory fall. But this lowering of the blood alkali is evidently not due to any such cause as an "anoxemic production of lactic acid," for throughout the first period no increase of lactic acid occurred in any of these experiments.

Turning now to the second period, that in which the inspired air contains less than 8 per cent of oxygen, we find very different phenomena and relations. The animals during this period are not merely making physiological responses to slight decrease of oxygen. On the contrary they are dying of asphyxia. The right hand ends of all the curves show the values for the various measurements at, or immediately before, death. During the



Figs. 1, 2 and 3. These figures show the course of events during progressive decrease of oxygen in three typical experiments. The two periods referred to in the text appear distinctly. In the first period the oxygen in the inspired air is above 8 per cent; in the second it is below 8 per cent. In the first over breathing is induced and the pH rises. From the curves for the CO₂ content and capacity, particularly in figures 1 and 3, it appears that this effect is due to the fact that decrease of the dissolved CO₂ is more rapid than the compensatory decrease of bicarbonates. The apparent discordance in figure 2 is probably due to slight errors of equilibration and analysis. During this first period the lactic acid undergoes no appreciable increase.

In the second period the pH, CO₂ content and capacity fall greatly and the lactic acid rises to a high figure. Death occurs at about 2 to 4 per cent of oxygen.

early part of this period the breathing is increased and the carbon dioxide content of the blood is correspondingly decreased. The carbon dioxide capacity, or blood alkali, falls rapidly to a figure far below normal, but not usually to a level that in itself would be fatal. Lactic acid appears in the blood in considerable, but not extremely toxic, amounts. The pH, which has risen in the first period, now falls considerably below normal although in no case to a level that by itself would necessarily be lethal.

In addition to these features there are two others, which are less evident, but even more important. In contrast to the increase of sensitivity noted in the period of anoxemia, the respiratory center during the period of asphyxia undergoes a progressive decrease of sensitivity. This development, which terminates in paralysis of breathing and death, is demonstrated by the lowered pH. Although the breathing may be greater than under normal conditions, its volume is nevertheless insufficient to keep the amount of carbonic acid in the blood down to equivalence with the decreased alkali. Hence the fall of pH. This fall shows that the respiratory center during asphyxia requires a progressively increasing stimulus to maintain its activity. In other words, the sensitivity of the center is steadily decreasing. If respiration were still equal to its duty, the over-breathing would be much greater than it is; and the relation of $[H_2CO_3]:[BHCO_3]$ in the blood, which determines the pH, would be kept normal in spite of the decrease of the alkali. This point will be more fully discussed in the next section.

The second important, but generally overlooked, development during the period of asphyxia is an exacerbation of the acapnia which is induced to a considerable extent even during the preceding period of anoxemia. In both periods carbon dioxide is thrown off faster than it is produced. This condition is easily overlooked and may have no great influence unless the supply of oxygen to the lungs is renewed in an effort to effect resuscitation. In that case, as we know from many other experiments in this laboratory, as soon as the depression of the respiratory center is partially relieved by a renewed supply of oxygen, acapnia makes itself felt in the lack of a stimulus adequate to induce breathing. Hence the life saving effect of inhalation of carbon dioxide diluted in air or oxygen after asphyxia. It supplies the requisite stimulus.

The sharp distinction here found between the first and second periods indicates that in the investigations of Gesell and others in which a considerable amount of lactic acid has appeared in the blood and the pH has fallen, the conditions were not those of a moderate decrease of oxygen within physiological limits, as in our first period; but were essentially the asphyxial and moribund conditions of our second period. It is probable, as Gesell believes, that acidosis develops in the center in asphyxia, as it does simultaneously in all active tissues under extreme deprivation of

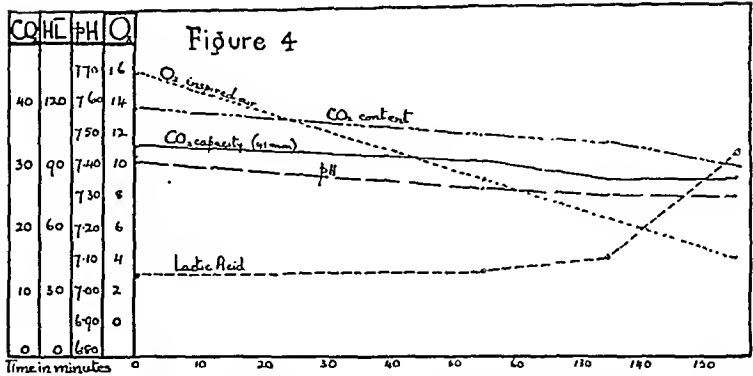


Fig. 4. This experiment, which was otherwise like the three preceding, shows the greatly decreased reactivity of an animal narcotized with a barbituric compound.

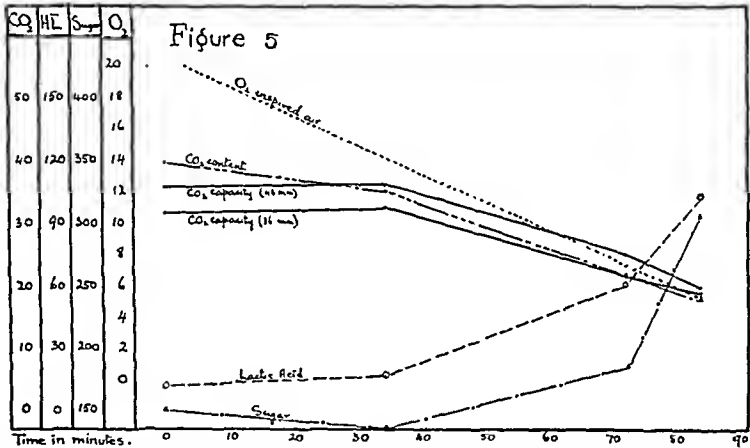


Fig. 5. In this experiment the conditions were like those in experiments 1, 2 and 3 except that both vagi had been cut.

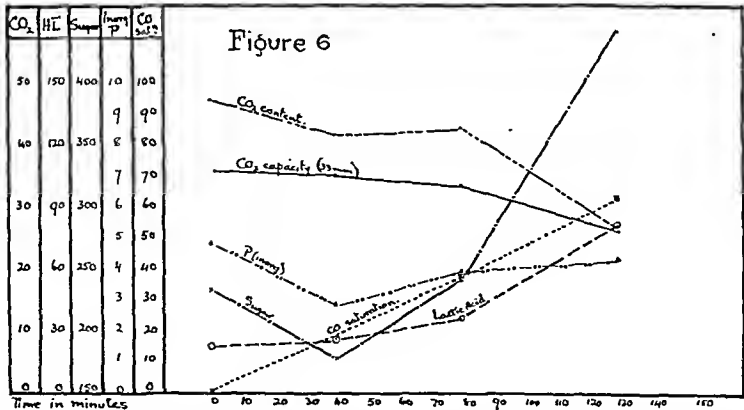


Fig. 6. In this experiment, instead of a progressive decrease of oxygen, the animal was subjected to a gradually increasing saturation of its blood with carbon monoxide.

oxygen. But our data show that this intracellular acidosis, instead of stimulating, is associated with a progressive depression of the respiratory and all other nerve centers. It terminates in respiratory failure and death.

In figure 4 are shown the data for an animal narcotized with amytal. The striking feature here is the almost complete absence of any response to decreased oxygen on the part of any of the functions observed; this is particularly the case in the first period of this experiment. Even in the second period the only distinct effects of acute asphyxia are an increase in lactic acid in the blood, a slight lowering of pH, and decrease both of the carbon dioxide content and capacity of the blood. In another investigation in this laboratory (Haggard and Greenberg (25)) a similar lack of change in the sugar content of the blood has been noted in dogs narcotized with amytal or other barbituric compounds. Evidently results in regard to respiration, blood gases and sugar metabolism obtained in experiments under this drug are to be regarded with doubt unless confirmed in other ways.

In figure 5 are shown the data from an animal in which both vagi were cut. The reactions of the functions here under study are seen to be not appreciably altered from those in animals with intact vagi. They are essentially the same as in the first three experiments above discussed.

In figure 6 are shown the data obtained from an animal in which deprivation of oxygen, leading to asphyxia and death, was induced by means of carbon monoxide. In this and other similar experiments the pure gas was prepared and was diluted down to a few tenths of 1 per cent with air. Unfortunately the pH was not determined. But it probably followed the same course as in the first three experiments above discussed. In respect to carbon dioxide content and capacity and lactic acid the effects were identical with those of the experiments in which the deprivation of oxygen was induced merely by rebreathing.

THE CRITICAL FACTORS IN ASPHYXIA. Ever since the conception of pH was introduced into chemistry, and from chemistry into biology and medical science, many physiologists and biochemists have made certain inferences from theory in regard to "acidosis," which account satisfactorily for some facts, but which largely ignore some others, no less important but of contrary implication. It is true that in asphyxia and related conditions lactic acid appears in the blood, the blood alkali is decreased, and the pH is lowered. It has been inferred therefore that in asphyxial acidosis and related conditions the body is intoxicated by acid. The part played by respiration has been largely misunderstood or ignored.

In many forms of acidosis the increase of lactic acid, or other acid elements, and the decrease of the blood alkali are really quite insufficient to cause such a perversion of pH as occurs. They are indeed insufficient to force any perversion whatever, if there were not some other factor also

acting. This statement is particularly true, as our data show, in asphyxial acidosis. The abnormal factor is depression of respiration: a depression too slight to be observed in any effect except a pH below normal.

Respiration has a sufficient "factor of safety" to compensate for very wide variations of blood alkali, and thus to prevent a lowering of pH. If in any way the blood alkali is decreased to a half, or a third, or a quarter of its normal value, it is only necessary for the dilution ratio of breathing—the volume of air breathed per unit mass of carbon dioxide exhaled—to be increased to double or threefold or fourfold: the pH would thus be held at its previous normal value. Respiration is capable of a much greater variation of activity than any such adjustment ever requires, short of such extreme conditions as in the Kussmaul breathing of diabetic coma (26). It follows therefore that, if respiration does not effect a complete compensation of an abnormally lowered pH, the cause lies in a depression of the sensitivity of the respiratory center. In other words, respiration is not maintaining a sufficient ventilation of carbon dioxide out of the blood in the lungs. The sensitivity of the respiratory center is depressed.

This conception has very great advantages for the analysis of many respiratory problems. It reduces the otherwise vague idea of the chemical control of breathing to two clear-cut factors: 1, the pH of the blood as the specific stimulus to the respiratory center, as postulated by Winterstein (11), and 2, the sensitivity of the respiratory center to this stimulus. Neither of these factors is adequate alone to account for the control of breathing. Both are necessary. Something of this sort is indeed now recognized; for instance, Peters and Van Slyke (27) say that the pH of the blood "shows whether the respiratory apparatus is operating in the normal manner to prevent any considerable change of the $[\text{BHCO}_3]:[\text{H}_2\text{CO}_3]$ ratio in the blood." But these authors consider that "the normal behavior of respiration does not necessarily entail the maintenance of a normal pH. When the bicarbonate of the plasma is reduced . . . pulmonary ventilation does not become accelerated enough to compensate entirely . . . so as to maintain pH unchanged."

This statement is well founded both experimentally and clinically. We suggest that it be amended only by substituting the words "basal pH" for "normal pH." Respiration maintains a basal pH only under basal conditions. Muscular exercise, anoxemia, mental excitement, asphyxia, ether anesthesia, morphine narcosis, and many other conditions, both normal and abnormal, alter the sensitivity of the center. Any increase or decrease of sensitivity to hydrogen ions induces a corresponding change in the pH of the blood. The pH of the blood is the expression and index of the sensitivity of the respiratory center.

According to this conception, the well established data concerning the effects of vigorous muscular exercise upon the ratio $[\text{H}_2\text{CO}_3]:[\text{BHCO}_3]$ in

the blood plasma should be interpreted as showing that the sensitivity of the center is increased during the exercise and decreased in the subsequent period of rest. The pH after exercise, as Douglas and Havard (28) have recently pointed out, is distinctly below the basal value; yet the subject breathes quietly. If the sensitivity of the respiratory center were constant at its basal value the high concentration of hydrogen ions in the blood during rest after exercise would induce vigorous hyperpnea.

With this conception in mind and largely on the basis of evidence to be referred to in the next section, we are led to conclude that the really critical factors in asphyxia are not the increase of lactic acid, nor the low pH of the blood, nor any other feature of acidosis. None of these conditions, nor all of them together, are sufficiently intense to account for the depression of respiration and other functions, and finally death, in asphyxia. The really critical factors are the depression of the sensitivity of the respiratory center and the acapnia, which has been induced by over breathing, and which leaves the depressed respiratory center with an inadequate stimulus. In other words, respiration does not fail, because the pH of the blood is so low that the system is poisoned by acidosis. On the contrary, it fails because the pH, although low, is not low enough to overcome the depression and to stimulate the center.

There is direct experimental evidence for this conception. Haldane (29) observed that animals, which have collapsed from carbon monoxide asphyxia, are partially revived when carbon dioxide is added to the atmosphere in which the asphyxia has been induced. Haggard (30) independently has shown that animals in an asphyxiant atmosphere of carbon monoxide reach a greater degree of saturation with that gas, before death is induced, when a considerable amount of carbon dioxide is also present in the atmosphere, than without it.

That asphyxial acidosis is a very different condition from the acidosis induced by intravenous injection of hydrochloric acid is shown by observations of Haggard and Henderson (31). They found that in the acidosis induced by intravenous injection of hydrochloric acid the inhalation of carbon dioxide is quickly fatal—exactly as the acidosis theory requires; but that two to three times as much lactic acid as hydrochloric acid, molecule for molecule, is required to induce acid poisoning. The body itself never produces such an amount.

RESUSCITATION FROM ASPHYXIA. There are strong clinical, as well as experimental, reasons for postulating variability in the sensitivity of the respiratory center, and for regarding depression of this sensitivity as one of the two critical factors in asphyxia. These reasons are afforded by experience in this laboratory in connection with the problem of resuscitation. For more than twenty-five years investigations in this laboratory have been devoted to the development of the method of resuscitation from post-

operative depression and other forms of asphyxia and acapnia by means of inhalation of carbon dioxide (32), diluted in air or in oxygen. In these investigations the fundamental ideas have been 1, to counteract the acapnia which, except in suffocation and drowning, always develops in asphyxia, and 2, to overcome the depression of the respiratory center by a super-normal stimulus. The inhaled carbon dioxide stimulates the asphyxiated center into activity; the oxygen, which this activity supplies, gradually restores a normal sensitivity in the center; the inhaled carbon dioxide then counteracts the acapnia which would otherwise render even a normally sensitive center apneic.

This idea has been applied successfully to resuscitation from carbon monoxide asphyxia (33), from post anesthetic depression (34), from asphyxia in the newborn (35), and from many related conditions. Depression of the respiratory center is the principal feature of drowning. Depression and the acapnia, which results from displacement of carbon dioxide from the bicarbonates of the blood by lactic acid in asphyxial acidosis, are the critical features in the asphyxia of the newborn. In these and other forms of asphyxia inhalation of carbon dioxide, diluted in air or oxygen, is now coming to be recognized as the specific means of resuscitation. It stimulates the respiratory center and counteracts acapnia. Experience for a decade past has proved its effectiveness for carbon monoxide asphyxia, and for post anesthetic and post operative depression (36).

In its various applications this method of resuscitation is now annually saving hundreds of lives. Yet if the conception that acidosis is the critical factor in asphyxia were correct, inhalation of carbon dioxide should certainly harm, not help. It should kill, not resuscitate. This method of resuscitation has in fact been repeatedly opposed on this theoretical ground. If the objection were based on facts, instead of an erroneous inference from the theory of the acid base equilibrium of the blood, then indeed it would be true as a recent, and quite logical, supporter of (37) the acidosis conception has expressed it, that "the use of carbon dioxide as a resuscitating agent in asphyxia neonatorum is not only superfluous, but may even be harmful, in that it tends to aggravate an already existing acidosis." Furthermore the alleged superfluousness and harmfulness of inhalation of carbon dioxide would apply equally to all other forms of asphyxia.

PRINCIPAL CONCLUSIONS

When the oxygen content of the inspired air is gradually reduced without accumulation of carbon dioxide, the effects develop in two distinct periods. One of these periods is that while the percentage of oxygen in the inspired air is above 8 per cent. The other period occurs after the oxygen has fallen below 8 per cent.

The first period involves merely anoxemia of tolerable degrees. Respira-

tion increases; the carbon dioxide content of the blood is decreased; and the pH rises. In compensation the blood alkali is automatically, but more slowly, decreased. In these reactions lactic acid plays no part. On the contrary, the amount of lactic acid in the blood remains at a normal level. The conditions induce, not acidosis, but alkalosis.

The second period is that of asphyxia and ends in death. The carbon dioxide content and capacity of the blood fall further; the lactic acid content is considerably increased; and the pH falls, with the development of acidosis.

The increase of breathing during the first period is clearly not due to formation of lactic acid and increase of the hydrogen ion concentration in the blood; nor is there any valid evidence for an acidosis localized in the respiratory center. The common, but erroneous, belief that moderate degrees of oxygen deficiency stimulate respiration through acidosis comes from observations on the acidosis which develops only under asphyxia. The belief is not valid even for this period; for under asphyxial acidosis the respiratory center is not stimulated but depressed. Moderate oxygen deficiency does not exert its influence upon breathing through acidosis and an increase of hydrogen ion concentration.

The conception of the chemical control of breathing here presented is as follows: The specific stimulus to the respiratory center is not carbon dioxide, but the hydrogen ion concentration of the blood plasma. The concentration of hydrogen ions is not an independent factor, but is the resultant of two factors, each of which controls a particular feature of respiration. The blood alkali determines the dilution ratio of breathing: that is, the volume of air breathed per unit mass of carbon dioxide exhaled. The carbon dioxide production of the body determines the volume of air breathed at the dilution ratio set by the alkali in use. But these controls operate to afford a basal pH only so long as the oxygen supply is ample and no other disturbing influences occur to alter the sensitivity of the respiratory center from the value that it has under basal conditions. The sensitivity sets the pH to which the respiratory center will respond and which it will therefore maintain.

Oxygen deficiency, through some non-acidotic process, increases the sensitivity of the respiratory center to its specific stimulus: the hydrogen ion concentration of the blood plasma. The center then maintains an increased volume of breathing which raises the pH of the blood. After muscular exercise to a slight degree and in asphyxia to an intense degree the sensitivity of the respiratory center is decreased. In consequence the volume of breathing is decreased below the amount, and the pressure of carbon dioxide and concentration of H_2CO_3 rise above the amount, that would correspond to the alkali in use in the blood. The pH of the blood is thus lowered. A restoration of normal sensitivity in the center and a

volume of breathing increased sufficiently to compensate for the decreased blood alkali would immediately restore a basal pH. Except under the most extreme variations of the blood alkali in disease, acidosis, in the sense of low pH, is always primarily evidence of depression of the respiratory center.

Chemically the pH of the blood expresses the balance of acids and bases. Emphasis upon this relation has tended to conceal another relation which is equally important. Physiologically the pH of the blood is an index of the sensitivity of the respiratory center.

BIBLIOGRAPHY

- (1) ARAKI, T. 1891. *Zeitschr. f. physiol. Chem.*, xv, 335; 1894, xix, 422.
- (2) BARCROFT, J. 1914. *Respiratory function of the blood*. Cambridge Univ. Press. 1925. *Respiratory function of the blood*. Part I. Lessons from high altitudes.
- (3) RYFFEL, J. H. 1910. *Journ. Physiol., Proc. Physiol. Soc.*, p. xxix.
- (4) HALDANE, J. S., A. M. KELLAS AND E. L. KENNAWAY. 1919. *Journ. Physiol.*, liii, 181.
- (5) HENDERSON, Y. 1919. *Science N. S.*, xlix, 431. 1920. *Journ. Biol. Chem.*, xliii, 29. 1932. *Nature*, cxxix, 649.
- (6) SINGER, W. 1929. *Zeitschr. f. gesamt. exper. Med.*, lxvi, 45; 1931, lxxviii, 712.
- (7) HESS, W. R. 1931. *Die Regulierung der Atmung*. Leipzig.
- (8) GESELL, R. 1925. *Physiol. Rev.*, v, 551.
- (9) GESELL, R., H. KRUEGER, H. NICHOLSON, C. BRASSFIELD AND M. PELECOVICH. 1932. *This Journal*, c, 202, 227.
- HALDI, J. 1932. *Ibid.*, xcix, 702.
- (10) LOEVENHART, A. S. 1915. *Arch. Int. Med.*, xv, 1059.
- (11) WINTERSTEIN, H. 1911. *Pflüger's Arch.*, cxxxviii, 167. WINTERSTEIN, H. AND GOLLWITZER-MEIER. 1928. *Pflüger's Arch.*, ccxix, 202.
- (12) GESELL, R., H. KRUEGER, G. GORHAM AND T. BERNTHAL. 1930. *This Journal*, xciv, 300, 339, 365, 387, 402.
- (13) MYERSON, H., J. LOMAN, H. T. EDWARDS AND D. B. DILL. 1931. *This Journal*, xcvi, 373.
- (14) HAYARD, R. E. AND P. T. KERRIDGE. 1929. *Biochem. Journ.*, xxiii, 600.
- (15) VAN SLYKE, D. D. AND J. M. NEILL. 1924. *Journ. Biol. Chem.*, lxi, 523.
- (16) FRIEDEMANN, T. E., M. COTONIO AND P. A. SHAFFER. 1927. *Journ. Biol. Chem.*, lxxiii, 335.
- (17) FOLIN, O. AND H. WU. 1920. *Journ. Biol. Chem.*, xli, 367.
- (18) FISK, C. H. AND Y. SUBBAROW. 1925. *Journ. Biol. Chem.*, lxvi, 395.
- (19) DALE, H. H. AND C. L. EVANS. 1920. *Journ. Physiol.*, liv, 167. *Ibid.*, 1922, lvi, 125.
- (20) LEVY, R. L., L. G. ROWNTREE AND W. McK. MARRIOTT. 1915. *Arch. Int. Med.*, xvi, 389.
- (21) JOHNSTON, C. J. 1928. *Journ. Biol. Chem.*, lxxix, 297.
- (22) BAYLISS, L. E., P. T. KERRIDGE AND R. C. VERNEY. 1926. *Journ. Physiol.*, lxi, 448.
- (23) JERVELL, O. 1928. *Acta Med. Scand., Suppl.*, xxiv-xxvi, 65.
- (24) MATHISON, G. C. 1910. *Heart*, ii, 54. 1911. *Journ. Physiol.*, xlii, 283.

- (25) HAGGARD, H. W. AND L. A. GREENBERG. Personal communication.
- (26) HENDERSON, Y. 1932. *Practitioner's Library of Medicine and Surgery*, New York. I. 751, Chapter xi. The principles controlling respiration in health and disease.
- (27) PETERS, J. P. AND D. D. VAN SLIKE. 1932. *Quantitative clinical chemistry*, i, 939. Baltimore.
- (28) DOUGLAS, C. G. AND R. E. HAVARD. 1932. *Journ. Physiol.*, lxxiv, 471.
- (29) HALDANE, J. S. 1922. *Respiration*. Yale University Press, 363.
- (30) HAGGARD, H. W. 1921. *This Journal*, lvi, 390.
- (31) HAGGARD, H. W. AND Y. HENDERSON. 1920. *Journ. Biol. Chem.*, xliii, 3.
- (32) HENDERSON, Y. 1906. *Brit. Med. Journ.*, ii, 1812. 1919. *Bull. Johns Hopkins Hosp.*, xxi, 235. 1908-1919. *This Journal*, xxi, 126; xxiii, 345; xxiv, 66; xxv, 310; 385; xxvi, 260; xxvii, 152; xlv, 533.
- (33) HENDERSON, Y. AND H. W. HAGGARD. 1922. *Journ. Amer. Med. Assoc.*, lxxix, 1137.
HENDERSON, Y. 1924. *Ibid.*, lxxxiii, 758; 1930, xciv, 179.
- (34) HENDERSON, Y., H. W. HAGGARD AND R. C. COBURN. 1920. *Journ. Amer. Med. Assoc.*, lxxiv, 783.
- (35) HENDERSON, Y. 1928. *Journ. Amer. Med. Assoc.*, xc, 583; 1931, xcvi, 495.
McILROY. 1927. *Lancet*, ii, 373.
WIENER, R. 1931. *Arch. Kinderheilkunde*, xcv, 65.
- (36) HENDERSON, Y. 1931. *Brit. Med. Journ.*, 687.
- (37) EASTMAN, N. J. 1932. *Bull. Johns Hopkins Hosp.*, l, 39.

STUDIES ON SUPRARENAL INSUFFICIENCY

XI. THE GROWTH OF TRANSPLANTED CORTICAL TISSUE IN THE RAT

LELAND C. WYMAN AND CAROLINE TUM SUDEN

From the Physiological Laboratory of Boston University School of Medicine and the Evans Memorial, Mass. Memorial Hospitals

Received for publication May 31, 1932

It has been conclusively demonstrated by Jaffe and Plavska (1926) and Jaffe (1927a) that free autoplasmic transplants of suprarenal cortex will grow and indefinitely maintain the health of suprarenalectomized rats and guinea pigs. Successful takes in rats had been reported by Poll as early as 1898, and some evidence that cortical transplants function in the rabbit had been presented by Busch, Leonard and Wright in 1908. Discussions of suprarenal transplantation may be found in the reviews by Jaffe (1927b) and Britton (1930). The technique for rats devised by Jaffe and Plavska has been used successfully by numerous investigators ever since 1926.

In spite of these facts the success of the earlier workers seems to be ignored by some recent writers, and such statements as the following occasionally appear in the literature: "Attempts to transplant the adrenal cortex have been made repeatedly, but on the whole the few 'takes' reported are open to criticism" (Johnson and Johnson, 1931), and "Auto-transplants of practically every tissue in the body have been attempted with much success. Auto-transplantation of the adrenal, however, has not met with such success" (Oldberg, 1929). Recent texts also disregard the work with rats and guinea pigs, or quote less conclusive experiments. *Human Physiology* by Winton and Bayliss (1931) contains this statement: "The complete proof of the specific secretory function of the gland would depend on the cure of adrenalectomized animals by transplanting cortical tissue from a normal animal; unfortunately, however, such grafting has been unsuccessful, the tissue degenerating in a short time."

During the past five years autoplasmic transplantation of the suprarenal cortex in rats by the technique of Jaffe and Plavska has been used successfully in this laboratory in a series of studies on suprarenal insufficiency. Both glands are removed, cut in half and the four parts transplanted into pockets in the abdominal muscle. From one to four of these transplants regenerate as masses of cortical tissue. It was noticed at autopsy that when one only of the transplants had regenerated it was almost invariably

larger than those seen when two or more had regenerated. Moreover, small size of regenerated transplants was often associated with the presence of accessory masses of cortical tissue within the abdominal cavity (the term "accessory" is used in this paper to refer to any gross mass of cortical tissue found within the abdominal cavity, irrespective of whether it may have regenerated from a fragment of the gland left at operation or from a true accessory cortical rest). This led us to suspect a physiological limitation with respect to the amount of cortical tissue which can regenerate from transplants or cortical rests. Accordingly a series of cortical transplantations made in various ways was studied and the results are reported below.

Autotransplantation. In order to get a figure which would express the relative amount of cortical tissue regenerating in transplanted or accessory masses three diameters of each mass found at autopsy were measured in millimeters; these were multiplied and the sum of these products for each animal was taken as an expression of the "volume" of regenerated cortical tissue present. It is realized that such a method is subject to considerable error, but the results showed that these figures were comparable, and that they roughly expressed what could plainly be seen at autopsy with regard to the relation of size of cortical masses to the number present. Data from transplantations done during the past five years were gathered in this way. In the recent experiments the cortical masses were also weighed in milligrams, and the weights were found to correspond with the figures obtained from the measurements.

Suprarenalectomy and transplantation were done on young rats from 45 to 90 days of age in 100 cases, and from 90 to 120 days of age in 43 cases. No correlation was found between the age at which transplantation was done and the growth of transplanted or accessory cortical tissue. Autopsies were performed during the third month after operation in 36 cases, during the fourth month in 57 cases, during the fifth month in 34 cases, and during the seventh, eighth and tenth months in the remaining 16 cases. Although the ranges for the amount of regenerated cortical tissue for those rats autopsied during the third and fourth months were the same, a greater percentage of the rats fell in the lower half of the range during the third month. The difference was not great enough to affect the curves for all the data. It is safe to say that regenerated cortical tissue is fully established by the fourth month, and from this time on there is little if any further growth.

A definite sex difference in the capacity to regenerate cortical tissue was found. Females regenerated over twice as much as males. The average "volume" for 69 female transplants was 47.8, and for 72 male transplants it was 20.5. The average weight for 31 females was 28 mgm., and for 29 males it was 11.6 mgm. This sex difference is shown in figures

1 and 2. It is well known that the suprarenal glands of female rats and mice are larger than those of males. Anyone who is familiar with this difference can usually tell the sex of a rat by looking at the suprarenals alone.

From one to four masses of cortical tissue may be present in a transplanted animal. These may be successful transplants, accessory masses

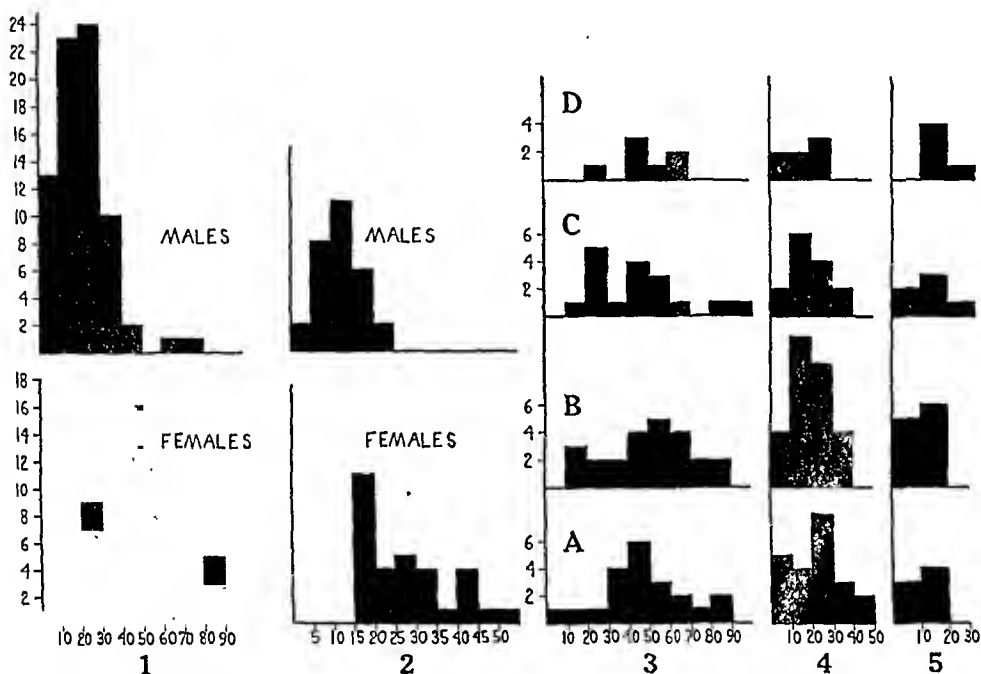


Fig. 1. "Volumes" (sum of products of three diameters; see text) of regenerated cortical tissue in 74 male and 69 female transplants. Abscissae represent "volumes." In this and in subsequent figures ordinates represent number of cases.

Fig. 2. Weights of regenerated cortical tissue in 29 male and 31 female transplants. Abscissae, weights in milligrams.

Fig. 3. "Volumes" of regenerated cortical masses in 69 female transplants. Abscissae, "volumes." In this and in figures 4 and 5: A, rats with one regenerated mass of cortical tissue; B, rats with two masses; C, rats with three masses; D, rats with four masses.

Fig. 4. "Volumes" of regenerated cortical masses in 71 male transplants. Abscissae, "volumes."

Fig. 5. Weights of regenerated cortical masses in 29 male transplants. Abscissae, weights in milligrams.

and transplants, or accessory masses alone, the transplants having disappeared. When one mass only is present it is large, sometimes fully twice as large as a normal suprarenal gland. When two or more masses are present they are correspondingly smaller. Often one or two very small transplants are accompanied by one or two larger accessory masses. On

account of the sex difference described above, figures for the two sexes have to be considered separately. Although there is considerable individual variation, figures 3, 4 and 5 show that in general the total amount of cortical tissue regenerating appears to be fairly constant, irrespective of the number of masses present. The data were obtained from 109 autoplasmic transplants made in the usual way, of which 23 had regenerated one transplant, 25 two transplants, 14 three transplants, and 11 four transplants, while 36 had one, two or no transplants together with one or two accessory masses. In addition 12 rats were deliberately transplanted with a single half gland and 22 received two transplants, each being half of a single gland. Of these latter 6 regenerated one and 16 regenerated both transplants.

Apparently in the rat there is a limiting factor, differing in the sexes, which regulates the amount of suprarenal cortical tissue which can regenerate from transplants, from fragments left at suprarenalectomy, or from cortical rests. This should be taken into consideration in studying the phenomenon of compensatory hypertrophy of cortical tissue. It may also have some bearing on the theory involved in experiments on the dosage of cortical extracts, inasmuch as it is probably a physiological or functional limitation. Obviously the transplantation method cannot be used to induce hyperfunction of the suprarenal cortex.

Autotransplantation and single suprarenalectomy. In three male rats, 48 days old, one suprarenal was removed and transplanted in two pieces into the abdominal muscles, and the other was left intact. At autopsy, 113 to 127 days later, no transplants were found. In five male and four female rats, 76 days old, one suprarenal was similarly transplanted, and a portion of the other was resected, leaving from one-quarter to one-half of the gland. At autopsy, 130 to 134 days later, no transplants were found and the portion of the suprarenal left in situ had regenerated into a mass about the size of a normal gland. Evidently the presence of one suprarenal, or even a good sized fragment of cortical tissue, completely inhibits the growth of autoplasmic transplants of cortical tissue. This is probably simply another expression of the physiological limitation with respect to growth of cortical tissue, and it may account for the failure of certain previous investigators to get successful "takes" when one gland was transplanted and the other was left to maintain the animal during the process of regeneration of the transplants.

Homotransplantation. Four male rats were suprarenalectomized at 38 and 45 days of age and each was transplanted with the suprarenal glands (in four pieces) from a litter mate. In all four cases the homotransplants regenerated and apparently functioned. At autopsy, 81 to 126 days after operation, it was found that one, three and four (two cases) of the four pieces transplanted had regenerated respectively.

In order to see if both autoplasmic and homoplasmic transplants would grow equally well in the same animal, one in the presence of the other, or if there would be a "preference" when both types of tissue were transplanted, ten male rats, 48 to 62 days of age, were suprarenalectomized and each was transplanted with one of its own suprarenal glands (in two pieces) on the right side of the abdomen and with a gland from a litter mate on the left side. At autopsy, from 87 to 121 days after operation, it was found that in six cases one or two autoplasmic transplants only had regenerated, and in four cases one or two of each type of transplant had regenerated. In these latter cases there was no apparent difference between the autoplasmic and the homoplasmic transplants. Evidently the presence of an autoplasmic transplant does not inhibit the growth of a homoplasmic transplant, and there is apparently no great "preference" when both types of tissue are offered.

Three female and four male rats, from 45 to 50 days of age, were transplanted in the abdominal muscles with four (4 cases) or 6 (3 cases) half suprarenal glands from litter mates, leaving their own glands intact. At autopsy, from 113 to 127 days after operation, no signs of these transplants were found. The rats' own glands were apparently normal. The presence of intact suprarenal glands inhibits the growth of homoplasmic transplants. This eliminates the possibility of studying excessive cortical function by means of "super-transplantation."

Jaffe (1927b) has reported that of fifteen rats examined one month after homotransplantation four had positive transplants.

Heterotransplantation. Whole suprarenal glands cut into two pieces from young and adult female mice were transplanted into the abdominal muscles of seven rats, 100 days old, immediately following suprarenalectomy of the host animals. Fragments of suprarenal cortex of various sizes from a rabbit and from a guinea pig were similarly transplanted into suprarenalectomized rats (seven cases for each species). In no case was there any evidence of growth or function of these heterotransplants, and at autopsy from 23 to 217 days after operation no signs of the transplanted tissues were found, the sites of insertion being perfectly clean.

Body growth. A study of the growth curves of 58 cases showed that the growth rate of suprarenalectomized rats having autoplasmic or homoplasmic transplants, or accessory cortical tissue was the same as that of normal rats of the same sex and age period. It was also seen that after the 80th or 90th day of life the growth rate of females decreased decidedly more than that of males. In 6 cases of suprarenalectomized males having no gross cortical tissue, during a period from the 90th to the 180th day of life, the growth rate fell to the normal female level. Except for these 6 cases, the data for the group as a whole agree closely with those given by Donaldson (1915).

SUMMARY

1. Suprarenalectomized female rats having autoplasmic transplants of cortical tissue, "accessory" cortical tissue, or both, regenerate more cortical tissue than do male rats.

2. The total amount of cortical tissue regenerating in such animals is fairly constant for each sex, irrespective of the number of masses present.

3. Homoplastic transplants of cortical tissue in rats are successful, and will grow in the presence of autoplasmic transplants made at the same time.

4. Heteroplastic transplants of suprarenal cortical tissue in rats are unsuccessful.

5. Neither autoplasmic nor homoplastic transplants of cortical tissue will grow in the presence of both, one, or even a good sized fragment of one of the animal's own suprarenal glands.

6. It is suggested that there is a physiological or functional factor limiting the growth of suprarenal cortical tissue in transplants or accessories. The transplantation method cannot be used to induce hyperfunction of the suprarenal cortex.

BIBLIOGRAPHY

- BRITTON, S. W. 1930. *Physiol. Reviews*, x, 617.
BUSCH, F. C., T. M. LEONARD AND T. WRIGHT. 1908. *Journ. Amer. Med. Assoc.*, li, 640.
DONALDSON, H. H. 1915. *The rat*. Memoir no. 6 of The Wistar Institute.
JAFFE, H. L. 1927a. *Journ. Exper. Med.*, xlv, 587.
1927b. *Arch. Pathol. and Lab. Med.*, iii, 414.
JAFFE, H. L. AND A. PLAVSKA. 1926. *Proc. Soc. Exper. Biol. and Med.*, xxiii, 528.
JOHNSON, A. AND V. JOHNSON. 1931. *This Journal*, xcvi, 392.
OLDBERG, E. 1929. *This Journal*, xci, 275.
POLL, H. 1898. *Zentralbl. f. Physiol.*, xii, 321.
WINTON, F. R. AND L. E. BAYLISS. 1931. *Human physiology*. Philadelphia.

CEREBROSPINAL ELASTICITY IN THE CAT AND MACAQUE

LEWIS H. WEED AND LOUIS B. FLEXNER

From the Department of Anatomy, Johns Hopkins University

Received for publication June 1, 1932

The possibility of applying with profit, to the dural sac and its contents, the customary physical formula for the determination of the coefficient of elasticity has been presented in former papers (1), (4). This formula is given as the quotient obtained by dividing the stress by the strain—i.e., the difference in pressure divided by the quotient of the difference in volume by the original volume, or $E = dP / \frac{dV}{V} = V \frac{dP}{dV}$. The E in this formula may be taken as a general coefficient of elasticity of the system as a whole and should not be confused with Young's Modulus (linear stretch), to which it can be simply related only in spherical or cylindrical systems.

The application of this general formula to the elasticity of the dural sac and its contents came through analysis of data yielded by experiments dealing with the abrupt tilting of dogs from the horizontal to the vertical (head-down, tail-down) positions. In one phase of this study, the pressure of the cerebrospinal fluid was determined by the bubble manometer (permitting measurement without dislocation of fluid) and by open-end manometers of different bore. It was found that as the bore of the manometer was increased the pressure-changes on tilting became less, though the volume of fluid dislocated into or from the manometer became greater. These differences in pressure-change (dP) and in volume-change (dV) were found to have a definite relationship to each other; the fraction dV/dP was ascertained to be of fairly constant value in any one dog or in those of the same size and age (4). With determination of the fraction dV/dP in any one animal and subsequent measurement of the intradural contents (cranial and spinal, for the total volume V), substitutions could be made in the formula $E = V \frac{dP}{dV}$ and the coefficient of elasticity established.

By this means, the coefficients of elasticity of a series of twenty dogs of different sizes and ages were computed and the results reported (1). The group of immature or juvenile animals was found to have a coefficient of elasticity which averaged 4.58×10^5 dynes per cm.²; the young adults, 4.22×10^5 dynes per cm.²; the adults, 4.03×10^5 dynes per cm.²; and the

obviously old animals, 3.81×10^5 dynes per cm.² In spite of the many difficulties of accurate classification of dogs according to age, the four groups showed great constancy in the value of the coefficient of elasticity and only one animal out of twenty yielded values not in keeping with the others of the same age-group.

The present report deals with the continuation of the study upon two other common laboratory mammals, the cat and the macaque. These animals were selected as the cat serves as a control to the findings on the dog, and as the macaque is essentially an animal of erect posture. The experiments were all performed as in the previous work, under ether anesthesia and with the tilting carried out so that the pressure of the cerebrospinal fluid could be recorded with open-end manometers of different bore. At the conclusion of the observations the animals were killed and the total intradural contents measured.

EXPERIMENTAL FINDINGS. With the determination of the pressure-changes on tilting to the vertical head-down and tail-down positions, and with the volume-changes derived from the calibrated manometers, it was possible to compute the fraction dV/dP for each manometer, after comparison with the volume and pressure-alterations recorded for the 1 mm. manometer. Four of these calculations were made from the data obtained in the head-down tiltings (manometers of 4, 6, 8 and 10 mm.) and similarly four calculations were made in the tail-down tiltings. Each of these series of four values was averaged, and finally a general average of all eight readings taken. Transient changes in elasticity (largely due to variations in the light surgical anesthesia considered most desirable) led to slight variations in the individual values of dV/dP for the different manometers but on the whole the results showed the same degree of constancy as was reported for the dog. As the coefficients of elasticity are somewhat different for the cat and the macaque, the findings will be presented separately for the two animals.

Cats. Table 1 gives data regarding the coefficient of elasticity of the dural sac and its contents in the series of 15 cats used. The fraction dV/dP ranged from 0.064 in an immature animal of 2220 grams to 0.117 in an adult of 2370 grams, while the coefficient of elasticity was found to vary between 3.86×10^5 dynes per cm.² in the same juvenile to 3.26×10^5 dynes per cm.² in an adult. The table shows a fair degree of constancy in the coefficient of elasticity within the various age-groups, the youngest group averaging 3.81×10^5 dynes per cm.²; the young adult group, 3.56×10^5 dynes per cm.²; the wholly adult group, 3.38×10^5 dynes per cm.² No obvious relationship between spinal length, body weight, intradural volume, and the coefficient of elasticity is apparent in the data comprising table 1.

In the cats, as in the dogs, the values of the fraction dV/dP were roughly of the same magnitude whether derived from data furnished by the head-

down tiltings or by tail-down tiltings. This similarity in the two values of the fraction was shown in many of the fifteen cats included in table 1, as for instance in cat C 8, where the head-down calculation of dV/dP was 0.100 and the tail-down, 0.098. Again, in cat C 57, the head-down fraction was 0.075 and the tail-down, 0.073; and in cat C 60, the head-down derivation yielded 0.077 and the tail-down 0.071. These three examples show a slightly larger fraction for the head-down tiltings than for the tail-down; for the whole series this generalization held as the average value of dV/dP obtained from data of head-down tiltings was 0.088 while that from the tail-down tiltings was 0.084. Occasional animals (as cat C 39) gave calculation of the fraction for the tail-down tiltings in excess of those of the

TABLE 1
Coefficient of elasticity of the dural sac and its contents in cats

EXPERIMENT NUMBER	WEIGHT	SPINAL LENGTH	INTRADURAL VOLUME	$\frac{dV}{dP}$	COEFFICIENT OF ELASTICITY	AGE-GROUP
	grams	mm.	cc.		dynes per cm. ²	
C 61	2,220	333	25.1	0.064	3.86×10^5	Immature
C 60	2,120	322	28.2	0.074	3.76×10^5	Immature
C 57	2,640	342	28.6	0.074	3.81×10^5	Immature
C 53	2,170	327	29.0	0.079	3.62×10^5	Young adult
C 51	2,480	358	28.7	0.082	3.45×10^5	Young adult
C 46	2,090	324	33.6	0.094	3.55×10^5	Young adult
C 9	2,220	325	35.2	0.095	3.65×10^5	Young adult
C 1	2,755	360	36.0	0.098	3.62×10^5	Young adult
C 8	3,420	340	36.4	0.099	3.62×10^5	Young adult
C 39	2,650	340	38.5	0.110	3.43×10^5	Young adult
C 42	2,380	345	28.4	0.085	3.29×10^5	Adult
C 50	3,060	382	30.8	0.086	3.53×10^5	Adult
C 54	3,070	352	31.0	0.094	3.26×10^5	Adult
C 56	3,750	376	37.3	0.108	3.40×10^5	Adult
C 48	2,370	408	40.6	0.117	3.42×10^5	Adult

head-down, and in this animal the difference was between 0.127 (for the tail-down) and 0.094 (for the head-down). One cat out of the fifteen constituting table 1 showed a head-down value of dV/dP double that of the tail-down (0.156 as compared to 0.078). This phenomenon of head-down values of a different magnitude than the tail-down was observed in one out of every five dogs, while in the cat series this single animal represented the only example.

It should be noted that the grouping of cats according to age presents difficulties of determination, even though inspection of the whole animal (teeth, hair, eyes, skin, bones, etc.), is resorted to. In the group of adults some old animals are possibly included but a frank decision of obvious

old age could not be made with certainty. The group of immature or juvenile animals was limited to those whose general size permitted the completion of the experimental procedures; smaller and still younger animals were apparently unable to stand the repeated tiltings in the anesthetized state. Were the series of animals to be extended beyond the numbers here reported, a gradual transition from one group to another would unquestionably be shown, as indicated by certain overlaps in the values of the coefficient of elasticity in the cats recorded in table 1.

It should be stated that table 1 includes all the cats used for these tilting experiments with four exceptions which for one reason or another were excluded from the tabulation. Thus, cat C 7 was not included as the animal had an outspoken respiratory infection and required artificial respiration throughout the period of experimentation. The other three cats (C 41, 49 and 58) were excluded because in each case only one tilting with manometers other than that of 1 mm. bore was made; these single tiltings were promptly followed by the death of the animal. It seems important to account for the exclusion of data from these four cats as the constancy in value of the coefficient of elasticity is definite in the fifteen cats in which the experimental procedures permitted gathering of adequate data for the determination of the value of the fraction dV/dP and of the total volume V . Only one of the animals (C 50) recorded in table 1 presents a coefficient of elasticity which quite obviously should place the animal in another age-group. In this case an animal classified as adult would fall, as far as the determined coefficient of elasticity is concerned, in the upper end of the group of young adults.

As a matter of record it seems of interest to include here the average pressure-alterations of the cerebrospinal fluid, as recorded by the 1 mm. open-end manometer on vertical head-down and tail-down tiltings, in order to give some idea of the protection of the central nervous system afforded by the bony coverings of the cranium and vertebral arches, against the full effects of atmospheric pressure. Report (2), (3) has been made that in dogs with an average spinal length (occiput to last lumbar spine) of 400 mm., vertical head-down tiltings gave an average increase of 104.9 mm. in the pressure of the cerebrospinal fluid (measured in the occipital region) while vertical tail-down tiltings were followed by an average decrease of 74.3 mm. Data obtained from similar tiltings are now in hand for twenty cats of different sizes and ages; the spinal length in this series averaged 349 mm. with extremes of 322 mm. and 408 mm. Head-down tiltings (61 carried out on the 20 animals) gave an average increase of 123 mm. in the pressure of the cerebrospinal fluid with highest reading of 171 mm. and lowest of 69 mm. The contrariwise tiltings (43 in number, performed on the same 20 cats) afforded an average decrease of 104 mm., with extremes of 151 mm. and 69 mm. The positional pressure-alterations of the cerebro-

spinal fluid are therefore of greater magnitude, in respect to spinal length, in the cat than in the dog; both of these laboratory mammals however show almost the same difference in reaction to the two vertical tiltings, the head-down pressure-change being approximately one-fourth greater.

Macaques. Ten catarrhine monkeys, all macaques, were included in this series; of these, four were *Pithecus rhesus* and six, the closely allied *Pithecus sinicus*. The experimental procedures were successfully carried out in all ten, so that no animals were excluded because of technical failures. Unfortunately all of the rhesus macaques were immature, juvenile animals, but the closeness of the two species makes inclusion of the ten animals in one series wholly permissible.

TABLE 2
Derivation of $\frac{dV}{dP}$ and E for macaque C 30

MANOMETER	HEAD-DOWN					TAIL-DOWN				
	Pressure-change, C.S.F.	Difference in pressure-change	Volume displaced	Difference in volume displaced	$\frac{dV}{dP}$	Pressure-change, C.S.F.	Difference in pressure-change	Volume displaced	Difference in volume displaced	$\frac{dV}{dP}$
mm.	cm.	cm.	cc.	cc.		cm.	cm.	cc.	cc.	
1	14.5		0.252			8.0		0.139		
4	10.8	3.7	1.048	0.796	0.215	6.0	2.0	0.582	0.443	0.221
6	7.1	7.4	1.874	1.622	0.219	4.0	4.0	1.056	0.917	0.229
8	4.7	9.8	2.397	2.145	0.219	2.6	5.4	1.326	1.187	0.219
10	3.6	10.9	2.565	2.313	0.212	2.0	6.0	1.425	1.286	0.214
Average.....					0.216					0.221

Average $\frac{dV}{dP} = 0.218$. Intradural volume = 82.6 cc. $E = V \frac{dP}{dV} = V / \frac{dV}{dP} = 3.74 \times 10^5$ dynes per cm.² (In C.G.S. units, dP = height in centimeters \times acceleration of gravity (980) \times density (1.006). Therefore $E = \frac{82.6}{0.218} \times 980 \times 1.006 = 3.74 \times 10^5$ dynes per cm.²).

One of the most important points at issue in this problem on the macaque was the value of the fraction dV/dP for the two types of vertical tilting, head-down and tail-down. The macaque, being a primate living for a large proportion of its life in the erect posture, should give an indication as to whether a specialized mechanism exists for the protection of the nervous system against postural hydrostatic effects of the fluid column within cerebral ventricles and subarachnoid space. In this series of ten animals, eight yielded results which showed a striking similarity in values between the fraction dV/dP derived on head-down tiltings and that on tail-down tiltings. Table 2 shows this identity of values for the two fractions; it

indicates the method of derivation of the fraction as well as its constancy in the repeated tiltings when the experimental conditions are adequate. One *Pithecus sinicus* (C 22) gave values of the fraction dV/dP for the head-down tiltings of more than double the magnitude of that for the contrariwise tiltings. A second bonnet macaque (C 26) yielded data which made the calculation of the tail-down fraction somewhat more than half that for the head-down tiltings. In each of these two cases, however, the head-down tiltings were of the approximate magnitude obtained in other animals for the two types of tiltings, and the head-down values of the fraction were therefore employed for the determination of the coefficient of elasticity. The occurrence of this phenomenon in two out of ten macaques may be compared to its occurrence in four out of twenty dogs and in one cat out of fifteen.

TABLE 3

Coefficient of elasticity of the dural sac and its contents in macaques

EXPERIMENT NUMBER	SPECIES	WEIGHT	SPINAL LENGTH	INTRADURAL CONTENTS	$\frac{dV}{dP}$	COEFFICIENT OF ELASTICITY	AGE-GROUP	PROBABLE AGE
		grams	mm.	cc.		dynes per cm. ²		
C 62	<i>P. rhesus</i>	2,150	242	80.4	0.154	5.15×10^5	Juvenile	21 mos.
C 64	<i>P. rhesus</i>	3,200	244	93.4	0.182	5.06×10^5	Juvenile	21 mos.
C 63	<i>P. rhesus</i>	2,285	234	74.8	0.154	4.79×10^5	Juvenile	31 mos.
C 65	<i>P. rhesus</i>	3,640	282	83.7	0.198	4.17×10^5	Juvenile	34 mos.
C 52	<i>P. sinicus</i>	3,050	260	74.9	0.171	4.32×10^5	Juvenile	40 mos.
C 55	<i>P. sinicus</i>	2,860	256	75.0	0.166	4.46×10^5	Juvenile	43 mos.
C 26	<i>P. sinicus</i>	3,900	312	81.7	0.197	4.09×10^5	Adult	63 mos.
C 34	<i>P. sinicus</i>	2,750	260	69.1	0.164	4.15×10^5	Adult	66 mos.
C 22	<i>P. sinicus</i>	6,250	348	92.0	0.228	3.98×10^5	Adult	8 yrs.
C 30	<i>P. sinicus</i>	5,200	325	82.6	0.218	3.74×10^5	Old	13 yrs.

With these exceptions, the values of the fraction dV/dP were of the same magnitude in the macaque whether calculated from the data of head-down tiltings or of tail-down, though the head-down values in the majority of animals were in slight excess of those from the opposite tiltings. The averages obtained from the two types of positional change from the horizontal were 0.180 for the head-down tiltings and 0.174 for the tail-down—a finding quite comparable to those in the cat and dog. Seven of the ten macaques showed this phenomenon but the other three animals yielded tail-down fractions dV/dP which were somewhat greater than the head-down derivations.

The determinations of the coefficient of elasticity for the macaques are given in table 3. The weights of the animals ranged from 2150 grams to 6250 grams, while the fraction dV/dP yielded its smallest value, 0.154,

in the youngest animal and its highest value, 0.228, in the heaviest (not the oldest) animal of the series. The coefficient of elasticity varied between 5.15×10^5 dynes per cm.² in the most juvenile of the monkeys to 3.74×10^5 dynes per cm.² in the oldest. Again, in these macaques as in the dogs and cats, there was no fixed relationship between body weight, spinal length, intradural contents, and the coefficient of elasticity.

The animals in table 3 are set down in order of age, as determined for the writers by Dr. Adolph H. Schultz, Associate Professor of Physical Anthropology in this institution. The decision as to relative ages was made on the basis of dentition (dental age), skull length and closure of cranial sutures; a maximum error of one-seventh in either direction may be applied to the ages recorded. Certain of the macaques are, as judged by the criteria employed, of almost identical age, particularly in the juvenile or immature group. Thus, animals C 62 and C 64 are both set down as of 21 months' age; the application of the maximum error of one-seventh might make either of these animals the older. Further application of the possible error would make some slight changes in the age-order of the animals in the table, but the order recorded represents the best judgment of age. The separation of the juvenile from the adult animals is in some ways an arbitrary one but it follows accepted anthropological usage. In all these determinations of age the greatest reliance has been placed upon the dental formula.

Analysis of the data in table 3 makes it apparent that the younger animals have coefficients of elasticity considerably higher than the adult and old animals. The coefficients of elasticity in the macaque series follow the age-grouping quite well, though one animal (C 65) classed as a juvenile shows a coefficient of elasticity which would place it among the adult animals. That one animal should fall out of series with the others in the experimental group is not surprising: rather is it surprising that such constancy in the coefficient of elasticity should be exhibited.

The experiments performed on this group of macaques gave information as to the extent of the pressure-changes in the cerebrospinal fluid, on tilting from the horizontal to the two vertical positions, when determined in the customary open-end 1 mm. manometer in the occipital region. Thirty-six tiltings to the head-down position in the ten monkeys gave an average pressure-increase of 106 mm. in the occipital cerebrospinal fluid, with 152 mm. recorded as the largest and 72 mm. as the smallest increase. The tail-down tiltings gave an average pressure-decrease of 80 mm. with extremes of 122 and 60 mm. The average spinal length (occiput to last lumbar spine) was 276 mm. in this group of ten macaques, the longest measuring 348 mm. and the shortest 234. These average pressure-changes in the occipital cerebrospinal fluid, in their relation to the average spinal lengths, may be directly compared to the similar pressure-alterations recorded for the dogs and cats.

DISCUSSION. The significance of the general coefficient of elasticity of the dural sac and its contents has been discussed in the previous reports of experiments on dogs (1), (4) and need not be repeated here. The general coefficient of elasticity of the cat is slightly lower than that of the dog, and shows similar age-changes. The cats may be taken to be of the same age-groups as the dogs but no obviously old cats were included. In the macaque series, the four *Pithecus rhesus* were unquestionably younger than any of the cats or dogs experimented upon while the six *Pithecus sinicus* represented all of the age-groups included in the dog and cat series and can therefore be directly compared to them. Taking the two juvenile specimens of the bonnet macaque for comparison with the immature dogs and cats, the average coefficient of elasticity of the dural sac and its contents is of the same magnitude in the dog and macaque, slightly lower in the cats. Similarly, the coefficients of elasticity in the young adults, adults and old animals are of the same values in the macaques and dogs, slightly less in the cats. All three of these mammals show in decisive fashion a gradual decrease of the coefficient of elasticity in dynes per cm.² (i.e., decrease in resistance to deformation) as one progresses from the juvenile into the adult and old animals. The occurrence of this phenomenon in these three laboratory animals suggests the strong possibility that it is of general biological significance.

Apart from any importance these observations may have in permitting calculations of unknown factors by substitution in the general physical formula employed, the experiments on macaques seem clear-cut in demonstrating that in this primate the general physiological mechanisms of pressure-adjustments about the central nervous system, in response to positional change, are not essentially different from those of the cat and dog. These two mammals may be taken to be typical four-footed animals in which the central nervous system is habitually carried in the horizontal position with only slight elevation of the head. The macaque, on the other hand, as has been pointed out, is an essentially erect animal, resting and usually sleeping in the vertical position though progression remains horizontal and four-footed. Had the assumption of the erect posture been accompanied by the development of protective agencies securing the nervous system against hydrostatic effects of the continuous column of the cerebrospinal fluid, difference in the reactions to tilting from the horizontal to the two vertical positions (head-down, tail-down) would be expected. First of all, the pressure-changes in the cerebrospinal fluid on such positional alterations would have been different in magnitude from those of the dog and cat. Yet the macaque showed on tilting to the head-down position an average pressure-increase of 106 mm. in the occipital cerebrospinal fluid and an average decrease of 80 mm. on the opposite positional change. With a spinal length considerably less than that of the dogs and cats used

(276 mm. as compared to 400 mm. and 349 mm. respectively), the average pressure-alterations in the macaque are the same as in the dog and slightly less than in the cat. Again, the general coefficient of elasticity (as calculated from the values of the fraction dV/dP) was found to be, in eight out of ten macaques, of the same magnitude whether derived from data furnished by head-down or tail-down tiltings. The exceptional animals which yielded coefficients of elasticity of almost double the magnitude from head-down tiltings as from the contrariwise positional changes, are encountered in the same proportion in dogs (one out of five) as in the macaque.

These two general characteristics of the reactions to tilting in the macaque indicate that the adoption of the erect posture has not been accompanied by the development of new physiological mechanisms for the protection of the nervous system against the hydrostatic pressures in the cerebrospinal fluid which the vertical position imposes upon the animal. Such protection as the bony coverings of the nervous system afford against the full effects of atmospheric pressure seems to be of the same degree in the macaque, the dog and the cat. The elastic elements within the bony cranio-vertebral canal also have coefficients of elasticity of the same general order of magnitude in the three mammals used: the coefficients for the macaque and dog are quite similar while that of the cat is slightly less.

It should be pointed out that these experiments have all been carried out on anesthetized animals and that there is of course a possibility of modification of the reactions due to this factor. As has been noted elsewhere (2), (3), such observations as have been made in unanesthetized man with lumbar pressures of the cerebrospinal fluid taken in the prone and sitting positions, indicate that the positional pressure-changes in this fluid are of the same relative magnitude in the unanesthetized as in the anesthetized state. It seems fair to conclude therefore that the data obtained from anesthetized mammals in these experiments may be accepted as portraying the animal's physiological reactions to positional change.

SUMMARY

The coefficients of elasticity of the dural sac and its contents have been determined in a series of 15 cats and 10 macaques, by application of the physical formula $E = dP / \frac{dV}{V}$. Deriving the fraction dV/dP from tilting experiments with measurement of the differences in pressure of the cerebrospinal fluid and in volume of fluid dislocated into or from manometers of various bores, the coefficients of elasticity were found to have the following values: in the macaques, from 5.15×10^5 dynes per cm.² in a very young juvenile to 3.74×10^5 dynes per cm.² in an old animal; in the cats, from 3.86×10^5 dynes per cm.² in an immature to 3.26×10^5 dynes per cm.² in an adult animal. The series showed a gradual decrease in the coeffi-

cients of elasticity from the immature to the old animals; in the various age-groups a fair degree of constancy in magnitude of the coefficients of elasticity was noted.

BIBLIOGRAPHY

- (1) FLEXNER, L. B., J. H. CLARK AND L. H. WEED. 1932. This Journal, ci, 292.
- (2) WEED, L. H. 1929. Intracranial pressure in health and disease. Chap. iii, p. 25 (Assoc. Research Nerv. & Ment. Dis., vol. viii, Baltimore).
- (3) WEED, L. H. 1929. Arch. Surgery, xviii, 1049.
- (4) WEED, L. H., L. B. FLEXNER AND J. H. CLARK. 1932. This Journal, c, 246.

ACTION POTENTIALS FROM SINGLE MUSCLE FIBERS

S. GELFAN AND G. H. BISHOP

From the Department of Physiology and Pharmacology, University of Alberta, Edmonton, Canada, and the Department of Ophthalmology, Washington University, and Laboratory of Applied Physiology, Oscar Johnson Institute, St. Louis, Mo.

Received for publication June 4, 1932

Adrian (1922) found, after stimulating small groups of skeletal fibers by the pore electrode, that the action potentials from these fibers varied in a discontinuous steplike fashion as the strength of stimulus was altered. The action potentials were proportional to the number of fibers activated, and apparently did not vary in a single fiber. Adrian therefore suggested that the all-or-none behavior of a muscle fiber is determined early in the chain of events from stimulus to response.

Since it has been demonstrated with microstimulation that normal single skeletal fibers can respond submaximally, depending upon the strength of stimulus (Gelfan, 1930, 1931; Pratt, 1930; Brown and Sichel, 1930),¹ the following question is raised: Is there an action potential present during a submaximal contraction of a single fiber, and does it vary with the degree of response of the single element?

Gelfan and Gerard (1930) showed that the gradations in contraction of a single fiber are not continuous from one end of the fiber to the other. The progressive spreading of the localized contraction from the point of stimulation, as the strength of stimulus is increased, is continuous only to a certain degree, after which a further increase in stimulus intensity elicits a complete and maximal response. These authors suggested that the submaximal responses may be due to a direct stimulation of some of the contractile units (sarcomers) by currents that are unable to initiate the conducted response. If that were true, an action potential might not be present during the submaximal contractions, since conduction of the response is absent. This would also answer the above-raised question. The following experiments were therefore undertaken to determine whether an action potential is present in single fiber during partial or submaximal contraction.

Diphasic action potentials were recorded by means of the cathode ray oscillograph both from sartorius and from the muscle fibers of the retro-lingual membrane, stimulated adequately for conduction to result, at a

¹ See also Fischl and Kahn, 1928, and Hintner, 1930.

sensitivity of the apparatus of 60 mm./mv., using microelectrodes, to establish a normal procedure for this technic. Such action potentials were in all respects identical with those recorded by Bishop and Gilson (1929) from the frog sartorius at threshold at 50 mm./mv. sensitivity, using fine steel wires as electrodes, the lead electrodes being placed accurately on the active fibers. Our present observations under the microscope indicate that such threshold responses were also due to single-fiber responses in the sartorius. In our present work, when the stimulus strength was increased, the action potentials increased by unit steps equal to the initial threshold response in amplitude. In the sartorius, the lead electrodes could be placed at first far enough apart to separate the two phases of the action potential. They were then brought closer together to ascertain whether, when the electrodes were separated by the distance that is permitted by the relatively short fibers of the retrolingual membrane, an action potential could be recorded at all from diphasic leads. Since the available length of fiber here is about 3 mm., an impulse conducting $2\frac{1}{2}$ m./sec. would give a record in which the two phases were superposed and of opposite sign after about 0.001 second, and at this time the first phase has reached only a fraction of its maximal value (fig. 3).

It was found that by resorting to a shunting device such reduction in amplitude of the diphasic record could be largely obviated. It was necessary in any case to arrange the electrodes and the muscle in the form of a Wheatstone bridge, to avoid the large shock artefact appearing when a stimulus of the order of one volt was applied at a distance of a fraction of a millimeter from one lead, with apparatus recording at the rate of 1 volt = 60 meters. The electrodes were first arranged with respect to the fiber (fig. 1) so that the resistance pathways in the tissue itself formed such a bridge, except that the balancing of the bridge was an extremely delicate procedure in the first place, and in the second, the balance changed both with movement of the fiber and with change of strength of stimulus. Two other bridge arms were therefore inserted, consisting of variable graphite-line resistances of the same order of magnitude as the stimulating cathode (100,000 ohms) arranged parallel to the stimulating electrodes as in figure 2. After initial approximate balance by movement of electrode 4, further compensation could be made as the stimulus was raised toward threshold by adjusting R5 and R6.

The virtues of this arrangement lie in the following secondary details: first, making the main bridge of the tissue itself and of the electrode resistances allowed the bridge to be approximately balanced for polarization at the same setting as for resistance, and without external bridge capacities. This was somewhat interfered with by the electrodes being of different sizes. Secondly, since electrodes 3 and 4 were grounded through resistances 5 and 6 about as effectively as was electrode 2, and since electrode 4

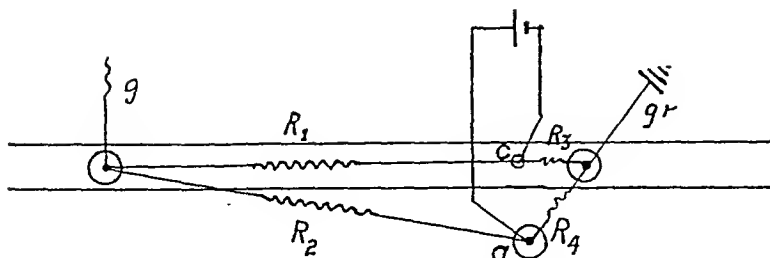


Fig. 1. Arrangement of electrodes on single muscle fiber for recording action potentials in response to stimulation. g , grid electrode; gr , ground electrode, leading to the amplifier of the cathode ray oscillograph; a and c , anode and cathode micro-electrodes for stimulating. The tissue resistances between these four electrodes can be considered diagrammatically as the four arms of a Wheatstone bridge. See text.

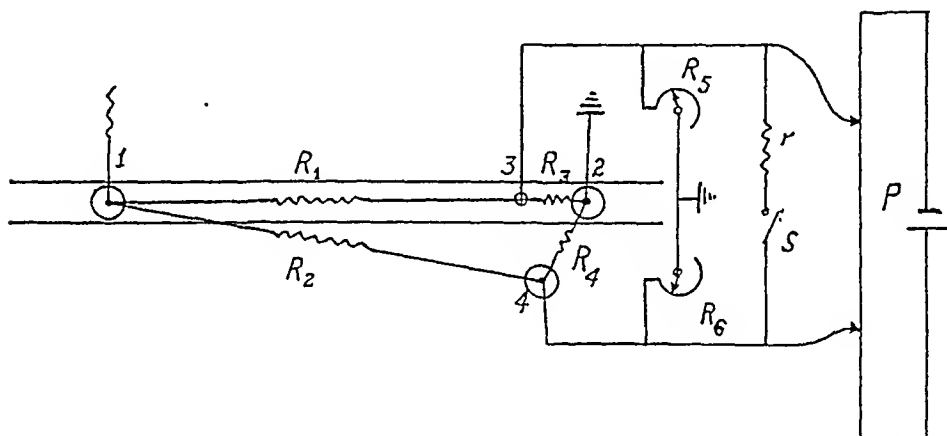


Fig. 2. Diagram as in figure 1 of single muscle fiber with leading and stimulating electrodes, 1 and 2, and 3 and 4, respectively, with the stimulating apparatus employed. P , potentiometer source of current; s , short-circuiting key which is opened to stimulate; r , protective resistance in series with S ; R_5 and R_6 , adjustable resistances in parallel with the two arms of the bridge R_3 and R_4 , for balancing bridge after approximate balance has been obtained by moving electrode 4. For effect of this arrangement on the action potential record see text.

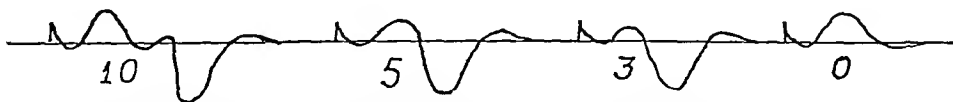


Fig. 3. Diagrams of the conducted potential records from a single muscle fiber at the surface of the frog sartorius, recorded in situ, with 10, 5, and 3 mm. distance between lead electrodes; and diagram, based on the previous ones, representing what the nonconducted action potential of a single muscle fiber would presumably have looked like if it had arisen under the stimulating cathode, but failed to conduct to the second lead electrode.

especially was a large one of low resistance, the result was a diffuse ground lead from these three electrodes in parallel, which materially reduced the amplitude of the record of the first phase of the action potential as compared to the second, thereby allowing their algebraic sum to be considerable even when superposed. On the other hand, if the second phase had failed to appear by reason of incomplete conduction, the first phase would have been of sufficient height to be clearly visible, as was demonstrated in the sartorius experiments where the leads could be placed far enough apart to separate the two phases in the record. At 3 mm. distance between electrodes 1 and 3, a separation just obtainable in the retrolingual membrane, a diphasic record showed a brief but detectable initial negative deflection followed by a much greater positive one (fig. 3). The whole might then have been interpreted as a single second phase were it not for the controls at greater separation.

After initial trials with condenser charges, galvanic currents from a potentiometer were used to stimulate for greater ease of compensation in the bridge. The potentiometer of 1,000 steps was built to have its capacity to ground about symmetrical with respect to the output leads (fig. 2). Battery current was allowed to flow continuously, the current through the bridge being turned on by breaking the short circuit *S*, a protecting resistance *r* of 25 ohms being insignificant as compared with bridge resistance. The interval of the opening of the short circuit could be varied from a small fraction of a thousandth of a second to about twenty thousandths by means of a rotating circuit breaker, or by a hand tap key for longer durations.

The electrode-tissue resistance was measured for high frequency and for direct currents (Bishop, 1929) to determine what complications polarization would introduce. The polarizable resistance of 25 μ electrodes proved to be several times their ohmic resistance, and was only partially reduced by plating with silver—silver chloride. The result is that even with a low constant voltage the current is at a maximum at first, then falls to a small fraction of this, in a time of the order of a sigma. The start of any galvanic current thus resembles a condenser charge or an induction shock in form, and for the rest of the current duration its effect may be insignificant. In spite of this, careful adjustment of threshold enabled us to obtain a few records late in the duration of the galvanic flow, after the initial distortion due to polarization had subsided. Such records were always diphasic, indicating all-or-none conduction, but this might have been due to the fact that current sufficient to stimulate at all would, if allowed to flow longer, mount to a stimulus adequate for complete conduction. If the current was now shortened in duration until it barely stimulated, the record was diphasic until both phases disappeared together, but then the break of the current with its concomitant distortion tended

to obscure the record. Shortening the duration of threshold currents usually required, however, no increase of strength for threshold until the end of the current fell within the polarization period of the start, indicating again that polarization promptly reduced the current led from a constant applied voltage to an ineffective value. Therefore the currents were usually shortened to a sigma or less, and the threshold regulated by the voltage, the stimuli then resembling rather long induction shocks, with their tails cut off.

One other complication entered. Many muscles would not respond to a stimulus below 1.3 volt with any duration (although some responded at less than 0.5 volt). At about 1.3 volt applied potential a sudden increase took place in the record of the stimulus distortion, and if the current was allowed to flow, bubbles arose from the electrodes. Apparently at about the dissociation potential difference for water an abrupt change takes place in the polarization phenomena at the electrodes, even before visible gas is evolved, and the distortion of the record associated with this is so large as to render further procedure impracticable. Increasing the size of the electrodes appeared to decrease somewhat the voltage necessary to stimulate, but this was limited by the fact that above 30 or 40 mu all threshold responses are conducted in an all-or-none manner, the voltage range for incomplete responses decreasing with increase in size of the stimulating cathode. An abrupt change in the character of the shock distortion in nerve stimulation is a common occurrence, even with induction shocks or condenser charges, and may presumably also be assigned to the passage of enough current across the electrodes, or perhaps across the tissue membranes, to initiate gas polarization by the dissociation of water.

In the muscle fibers of the retrolingual membrane of the green frog, we were unable to detect any record of excitation potential similar to a normal muscle action potential, from an incompletely conducted, i.e., submaximal, response. In some cases a difference of a few millivolts in threshold determined consistently whether a diphasic action potential or none at all was elicited. In others, the threshold rose slowly but progressively, even though stimulated only about once per two seconds. It might be argued here that with a small response of the fiber, only in the region of the stimulus, a weakened single first phase would not have been detectable at the sensitivity employed. However, under conditions where less than one per cent change in stimulus determined the character of the response, it seems reasonable to suppose that an impulse localized under the electrode, and due to a stimulus just subthreshold for conduction, would not be far below the conducted impulse in intensity, if it were otherwise of the same character. The fact that the break between the full-sized potential of the conducted response and its apparently total absence in the case of the nonconducted one is so complete and so sharp, even with

accurately graded stimuli, indicates that the two responses are different in type; that is, with respect to the electrical manifestations of response. Aside from the matter of conduction, the *mechanical* responses may be similar.

In two experiments fibers were encountered where marked *treppe* occurred. With the interrupter running at 2-second intervals the stimulus voltage was raised to just threshold for a conducted response. It could then be gradually lowered by as much as 20 per cent with the fiber responding each time all-or-none. If a few seconds' interval were then allowed without stimulation, the threshold had returned to near its initial value. This degree of *treppe*, reminiscent of the usual condition in the vertebrate heart, is much greater than that sometimes showing in fatigued nerve. That these muscles were not more than temporarily fatigued was indicated by the fact that the series could be repeated on the same fiber again and again, and by the further fact that the action potential record did not materially fall off during the procedure as it does, parallel to the tension, in normal fatigue. Neither, however, did the potential rise noticeably as the fiber became more irritable. This is a *treppe*, then, at least in the sense of progressive increase in irritability with activity, if not an increase in response. We do not know whether the contractions altered in tension developed, although all-or-none contractile responses of single fibers as recorded by the mercury droplet method, do exhibit the staircase phenomenon (Pratt and Eisenberger, 1919, fig. 30).

As far as could be judged visually, under the microscope, the duration of the nonconducted responses appeared quite comparable to the conducted ones. It is difficult to determine whether the spread of the submaximal contraction in the single fiber, as compared to the extremely localized minute twitch under the electrode, constitutes a *partial* conduction of the contractile wave. Lillie (1929) has pointed out that in the passive iron wire, a fine scratch, that is, activation of only a small surface area, does not evoke the transmission of the response. The very slight spread in this case quickly decrements to zero. In the muscle fiber, however, though the spread of the submaximal response is limited, it is considerable. If the response is a partially conducted one, it is reasonable to expect, from the above considerations, that the presence of a decremented action potential would have been detected in our experiments. On the other hand, if the localized responses are due to a direct activation of the contractile elements of the fiber, without at the same time initiating the propagated response, it might be assumed, as Gelfan and Gerard (1930) have done, that the spread of the localized response is due to the spread of the localized stimulus.

In two experiments with the retrolingual membrane, the fibers failed to give a conducted response at less than $4\frac{1}{2}$ volts applied potential, with any

duration of stimulus. They gave good nonconducted responses at somewhat less than this, but not with short durations of stimulation of the order of sigma. Stimuli of longer duration were therefore applied by means of a tap key worked by hand. It was then observed that the responses lasted as long as the stimulus, not as a tetanus, but as a nonconducted *contracture*. These fibers thus differed from normal ones not chiefly in ease of eliciting nonconducted responses, but in the high threshold for all-or-none response, such that the range of stimulus strength over which the incomplete response could be obtained was extremely wide.

This is obviously the idiopathic galvanic contracture characteristic of muscle in poor condition or of invertebrate muscles. Now maintaining the strength of stimulation below that required for conducted responses, different durations of current were applied. Here all succeeding responses must have been local ones and of the character of contracture rather than of propagated impulses. The object was to determine whether indubitable contractures could relax so rapidly after a brief stimulus as to be indistinguishable in duration from twitches, conducted or not, such as had been observed in normal muscles. Visual observation under the microscope would at times impress the observer that these contractures were more sluggish and had a longer relaxation period, as compared to the localized response of a normal muscle fiber. The difference in duration however, if any, between brief contracture responses and conducted ones, could not be definitely determined by visual inspection. This aspect of the problem will be further investigated photographically.

We may, therefore, provisionally conclude that the submaximal contractions of a single skeletal fiber, what have here been termed nonconducted or partially conducted responses, differ from completely conducted ones in lacking a normal action potential. Since the duration and other characteristics of these contractions cannot be definitely distinguished, by the means so far employed, from responses that are short contractures, it is conceivable that they may be of the nature of brief contractures. Their twitchlike brevity is permitted by the briefness of the stimulus, whether this be an induction shock or galvanic current promptly blocked by the polarization of the small electrodes that must be employed. It is possible, when stimulated by a current of the order of duration of the muscle action current itself, that a local contracture may take place of the order of duration of the normal muscle all-or-none contraction. Such a viewpoint would not presume any fundamental difference, so far as may be detected by the methods employed, between a muscle contraction and a muscle contracture, except that one appears to be initiated by the muscle action current, the other by current from a battery. This is consistent with the viewpoint taken by Gasser, in defining contracture (1930, p. 36), that "only conduction of the mechanical response and a wavelike action

potential are missing." How significant this slight difference might be for an interpretation of the physiological action of muscle, however, only further investigation may decide.

SUMMARY

1. A method is described that permits cathode ray oscillograph measurements of action potentials from single muscle fibers when the latter are activated by microelectrodes to give conducted responses.

2. In the single muscle fiber of the retrolingual membrane, no action potential could be detected during submaximal contraction.

BIBLIOGRAPHY

- ADRIAN, E. D. 1922. *Arch. Nécr de Physiol.*, vii, 330.
BISHOP, G. H. AND A. S. GILSON, JR. 1929. *This Journal*, lxxxix, 135.
BISHOP, G. H. 1929. *This Journal*, lxxxix, 618.
BROWN, D. E. S. AND F. J. M. SICHEL. 1930. *Science*, lxxii, 17.
FISCHL, E. AND R. H. KAHN. 1928. *Pflüger's Arch.*, ccix, 33.
GASSER, H. S. 1930. *Physiol. Rev.*, x, 35.
GELFAN, S. 1930. *This Journal*, xciii, 1.
1931. *Ibid.*, xevi, 16.
GELFAN, S. AND R. W. GERARD. 1930. *This Journal*, xcv, 412.
HINTNER, H. 1930. *Pflüger's Arch.*, cciv, 608.
LILLIE, R. S. 1929. *Journ. Gen. Physiol.*, xiii, 1.
PRATT, F. H. AND J. P. EISENBERGER. 1919. *This Journal*, xlix, 1.
PRATT, F. H. 1930. *This Journal*, xciii, 11, 680.

THE BLUE EXCITATION CURVE OF DICHROMATS

ELLIS FREEMAN AND W. F. HAMILTON

*From the Department of Psychology, and Department of Physiology and Pharmacology,
University of Louisville*

Received for publication June 4, 1932

This investigation aims to find the characteristics of dichromatic or color-blind vision which may be a check upon the data of the trichromatic functions of the normal eye described in an earlier paper (1). The method of making the matches to spectral monochromes and the apparatus were the same as those employed for the normal.

In table 1 the first pair of subjects are protanopic, and are represented by the lower curve in figure 1 for matches between 660 and 500. The second pair are deuteranopic, and are represented by the upper curve for the same range. All four subjects, since they have identical blue functions, are represented by the same curve for matches between 517 and 460.

In our paper on normals the results differed from the classical data in that our mixtures were as saturated as the monochromes which they matched as far down as 517. Classically an obvious loss of saturation has been described in mixtures which match monochromes up to 570 or higher, and the blue excitation curve has been extended deep into the long-wave region of the spectrum to represent this phenomenon. In attempting to account for the difference between our results and those of König, Abney, Wright, Guild, and others, we have found a sufficient general explanation in the state of adaptation of the eye. Without exception the experimental procedures suggest that, when the loss of saturation in this region occurred, the subject was adapted to an intensity below the intensity of the stimulus. In our work, where the loss did not occur, the subject was adapted to brightness comparable to that of the stimulus,—the condition of all ordinary useful vision. Thus our work on normals pointed to the likelihood that the loss, in the observations of others was due to desaturating scotopic factors introduced by dark-adaptation.¹ It also

¹ Whereas light-adaptation and the other factors discussed in our previous paper may be a complete explanation of the divergence between the classical and our results, it should be further noted, for what it may be worth, that tentative observations designed to elucidate the rôle of field size have produced desaturations even in the presence of light-adaptation. These occur simply when the fields are viewed through an ordinary scale-reading telescope placed at the eye-piece of the original apparatus. With the telescope so placed, desaturation seems to be independent of field size, intensity, and stray light.

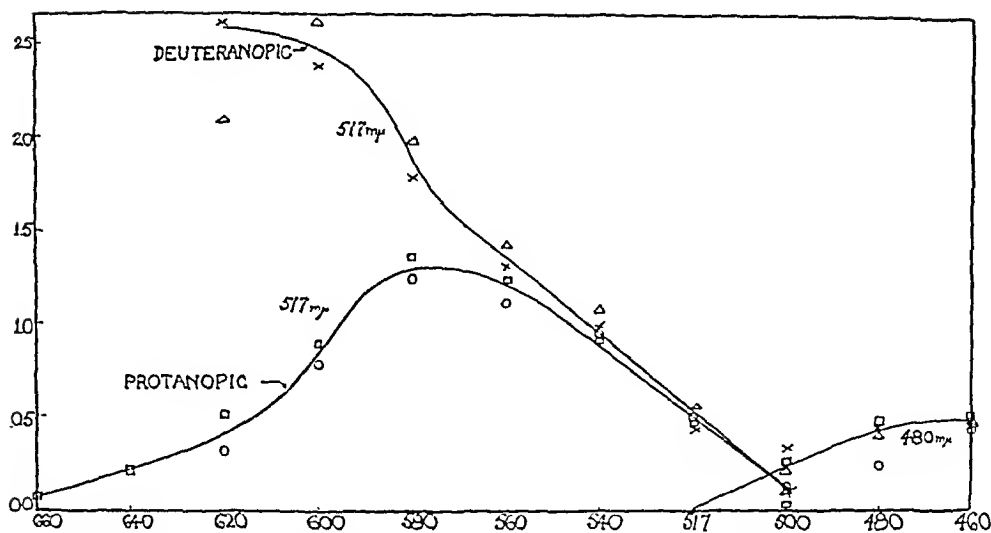


Fig. 1. Excitation (spectral color-mixture) curves of two types of dichromats. Wave-length in $m\mu$.

TABLE 1

Showing spectral color-mixture matches by two types of color-blind subjects

The first type (2 subjects) possessing a protanopic curve, and the second type (2 subjects) possessing a deuteranopic curve. The values are given in millimeters of slit width for each of the primaries required to match the monochrome.

SYMBOL OF SUBJECT	MONO-CHROME MATCHED, $m\mu$	PRIMARIES		SYMBOL OF SUBJECT	MONO-CHROME MATCHED, $m\mu$	PRIMARIES	
		517 $m\mu$	480 $m\mu$			517 $m\mu$	480 $m\mu$
Square	460		0.48	Triangle	460		0.48
	480		0.46		480		0.44
	500	0.01	0.24		500	0.10	0.28
	520	0.47			520	0.50	
	540	0.91			540	1.07	
	560	1.23			560	1.42	
	580	1.36			580	1.97	
	600	0.89			600	2.61	
	620	0.51			620	2.09	
	640	0.21			670	2.21	
	660	0.07					
	670	0.05					
Circle	460		0.42	Cross	460		0.48
	480		0.23		480		0.43
	500	0.12	0.27		500	0.11	0.32
	520	0.49			520	0.45	
	540	0.94			540	0.97	
	560	1.11			560	1.30	
	580	1.24			580	1.79	
	600	0.78			600	2.38	
	620	0.31			620	2.61	

indicated that a set of excitation curves representing photopic vision possesses a blue curve that falls to 0 in the region of 517.

Since the characteristics of partial color-blindness arise from the apparent absence of either the red or the green process, we should expect the corresponding excitation curves to indicate this fact, as they do. The blue curve, on the other hand, is like that of the normal. This conformity of blue in normals and in dichromats offers a simplified means of making a check on the results obtained with the normals.

In making the complex matches of the normals above 517, the remote possibility remained that very small amounts of desaturation in the mixed field had been neglected. The color-blind eye, since it involves fewer variables, offers a simple way of determining the extent of the blue curve. Since we have found the blue curve for the color-blind to coincide with that of the normal, it constitutes an important argument against the remote possibility that the blue curve had not been extended sufficiently far towards the red in the case of the normals.

The greater simplicity of determining the excitation curves of the color-blind lies in the circumstance that matches above the vicinity of 517 can be made with only one primary. Thus the color-blind had no more difficulty in making a "heterochromatic" match than the normal would have in matching two fields of the same wave-length. The fact that this was true when matches were made between 517 and longer wave-lengths shows conclusively that in the region of 517 there is no blue excitation.²

Not only is it simpler to determine the excitation curves of the dichromat, but also it is easier to determine his wave-length discrimination curves. Steindler (2) has described the "hue" discrimination curve of the deuteranope as consisting of a single region of low threshold near 500. This has been corroborated by other investigators (3). In contrast with Steindler's complex curve for the protanope, however, a simple curve has been found (3), (4), identical in all respects with the curve of the deuteranope. Evidently, as has been suggested (4), Steindler allowed her subjects to mistake brightness differences for hue differences. The identity of the wave-length discrimination curves for the two types of dichromats is consistent with, and to be expected from, the coalescence of the two types of "long" excitation curves of figure 1 in the vicinity of and below 517. Of course, the red and green excitation curves of the normal show no such tendency to come together here (1).

The fact that wave-length discrimination is confined to a restricted region in the blue-green and that it is entirely lacking above the region of 517, in the color-blind, justifies still further the restriction of the blue curve to below the region of 517 in the normal.

² This is subject to the assumption that the blue curve cannot be proportional to both the protanopic and deuteranopic "long" curves at all wave-lengths (1).

SUMMARY

Considerations of color-mixture and of wave-length discrimination in the two types of dichromats indicate, as do the data for the normal eye, that the blue excitation curve should not extend above the region of 517.

BIBLIOGRAPHY

- (1) HAMILTON W. F. AND E. FREEMAN. *Journ. Optical Soc. America*, June 1932.
- (2) STEINDLER. *Sitzungsb. d. Wiener Akad.*, 1906, cxv, ii A, 39.
- (3) In press.
- (4) LAURENS, H. AND W. F. HAMILTON. *This Journal*, lxx, 1923, 547.

CATALEPSY CAUSED BY LESIONS BETWEEN THE MAMMIL-LARY BODIES AND THIRD NERVE IN THE CAT

S. W. RANSON AND W. R. INGRAM

From the Institute of Neurology, Northwestern University Medical School

Received for publication June 6, 1932

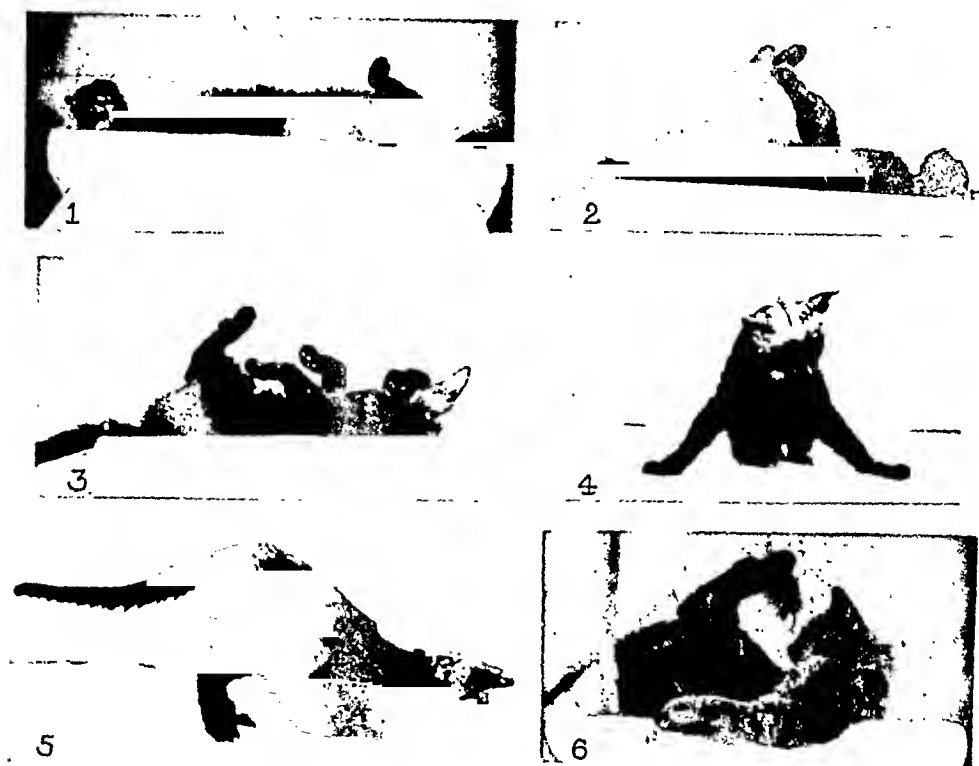
With the Horsley-Clarke apparatus and a bipolar needle electrode we have placed small bilateral electrolytic lesions in the region between the caudal border of the mammillary bodies and the rostral fibers of the third nerve. This work was undertaken as an extension of an investigation of the functional disturbances caused by the destruction of the red nuclei, and the technique employed has been fully discussed in the report of those experiments (Ingram and Ranson, 1932). In the course of those experiments it had been noted that cats, in which the lesions had extended some distance rostral to the third nerve, exhibited an apathy and inertia quite striking when compared with those in which the lesions did not extend so far forward.

OBSERVATIONS. The investigation was renewed in order to determine the effect of lesions situated just rostral to both red nuclei. Many of the cats in this series were very lethargic and required rather strong stimuli to wake them up. They would maintain for long periods of time any posture in which they happened to be placed. Their behavior was so different from that of cats with lesions more caudally placed in the red nuclei that our attention was at once directed to their cataleptic state.

In five of the eight cataleptic cats the lesions had been made under ether anesthesia and without aseptic precautions. Within three or four hours they had recovered from the anesthetic sufficiently to permit examination. They were examined again six hours after the operation and again at the end of twenty-four hours, when they were killed to prevent the results being complicated by infection. Within three or four hours all of these cats could stand and walk. They were, however, disinclined to walk and would never take more than a few steps at a time and would come to rest as soon as they were permitted to do so. If when they were lying flat on their side the tail was pinched, they could readily get up into a standing position. But if left undisturbed they would lie quietly and apparently asleep.

They exhibited an increased muscle tonus of an extremely plastic type, by virtue of which they would assume and maintain any posture in which

it pleased the experimenter to mold them. The plasticity could be especially well demonstrated when the cats were resting on their backs in a shallow trough (figs. 1 and 2). If the limbs were passively flexed they remained flexed. If they were passively extended they remained extended indefinitely, and considerable force was required to return them again to the flexed position. In spite of this marked resistance to flexion, which was as great as in the best decerebrate preparations, the limbs when they



Figs. 1, 2, 3, 4, and 5. Cataleptic cat three days after bilateral electrolytic lesions had been made caudal to the mammillary body. On the first day this cat would take and hold the position shown for another cat in figure 6, but on the third day it would only hold its head up as shown in figure 3.

Fig. 6. Cataleptic cat four hours after bilateral electrolytic lesions had been made caudal to the mammillary body.

had once been passively flexed exhibited no tendency to extend themselves. These cats differed from decerebrate preparations, whether made by the transection or anemic methods, for according to our experience the limbs of a decerebrate cat showing a comparable degree of resistance to passive flexion will not remain flexed for any considerable period of time but will always return gradually to the fully extended position. In this respect these animals were much more plastic than any decerebrate preparations which we have examined.

This plasticity also involved the muscles of the neck, trunk, and tail. If the head and pelvis were raised from the trough they would remain elevated for a time (figs. 3 and 6), but this posture was one which could not be maintained for more than a minute. The head and pelvis would gradually yield to gravity and sink back into the trough.

The animals could be molded in various standing positions (figs. 4 and 5) and would maintain them for long periods. They have been observed to stand in these absurd postures for as much as fifteen minutes at a time. A stimulus, such as pinching the tail would, however, wake them up and cause them to assume again a normal posture and take a few steps, after which, if left alone, they would apparently go to sleep again in the standing position.

So far as could be judged by their behavior these animals were very drowsy and slept most of the time. They seemed to sleep equally well on their backs in the trough or when standing on their feet. They paid no attention to ordinary stimuli such as sounds or stroking of the hair but could be aroused by pinching the tail. The threshold for painful stimuli was raised so that stimuli, which would ordinarily obtain a prompt and decisive response, would have to be repeated or increased in intensity to arouse the animal. Apparently their readiness to maintain unusual and rather uncomfortable postures was directly related to this somnolence, and the paucity of voluntary movements was an important factor in the ease with which plasticity would be demonstrated.

When lying on their backs in the trough with limbs extended or when supported in a canvas hammock with the limbs pendant, the cats offered considerable resistance to passive flexion of all four legs. This could readily be demonstrated whether the flexing force was applied above the wrist or ankle or whether it was applied to the pads of the toes in the position of the positive Stütz reflex. As has already been intimated these cats differed from decerebrate preparations in that when lying on their backs in the trough, there was no tendency for the limbs to take on of themselves an extended position though, when passively extended, they would retain this position with considerable strength. Furthermore, these cataleptic cats differed from decerebrate preparations in their ability, when properly aroused, to get on their feet, stand and walk. If, when lying on their backs in the trough with the limbs extended in the air the trough was tipped so that they fell over on their side, they would wake up, get on their feet, and perhaps take a few steps and then apparently go to sleep again in the standing position. Sometimes under such conditions the contraction of the muscles involved in maintaining a standing position would be gradually overcome by force of gravity; the head would droop until it touched the floor, the limbs would sag and occasionally assume sprawling positions until finally the cat was lying, asleep, often in odd

postures. If the animal went to sleep with the head and fore quarters hanging over the edge of the table it would gradually slip off and fall, waking up only when it struck the floor.

A normal cat will seek out a comfortable spot before lying down to sleep; but our cats exhibited a more or less persistent somnolence which caused them to fall asleep in almost any position. Although they were not comatose, because they could be aroused and would then react in a fairly normal manner, their sleep could not be regarded as normal. It resembled in many respects that seen in patients with encephalitis lethargica.

The cats would eat, if food was held to their mouths, but in quite an automatic, almost reflex manner, without particular interest.

In most instances the pupils were widely dilated indicating an involvement of the Edinger-Westphal nucleus. In one instance both pupils were constricted and in this case the lesion must have been situated rostral to that nucleus. The eyes were usually held closed, which may have indicated a ptosis due to paralysis of the third nerve or a contraction of the orbicularis oculi.

In several cats the lesions were placed under aseptic conditions. Three of these were cataleptic. One remained in this state for seven days but died on the eighth day of pneumonia. Another remained cataleptic for three days. The fourth day no observations were made. On the fifth day it was wide awake and active. The third cat remained cataleptic for only two days after which the somnolence disappeared. In these two animals, after the lethargy had disappeared and they had again become alert and active, the plasticity also disappeared and they could no longer be posed in unusual postures but would again voluntarily assume normal attitudes.

Microscopical sections of the brains from these animals showed that in all cases the lesions were bilateral and were located in the region between the mamillary body and the third nerve. Usually two and sometimes three small lesions had been placed one rostral to another on each side of the midline and were more or less confluent rostrocaudally but not across the midline. This confluence produced in effect a single irregular lesion on each side of the midline about three millimeters long and one-half to one millimeter wide. Although there was great variation in the shape and size of the lesions and in the structures which were destroyed, it may be said in general that the lesions centered around the point where the habenulopeduncular tract approaches the ventral surface of the brain stem before turning caudally toward the interpeduncular nucleus. A study of the sections does not enable us to say what structure it was, the destruction of which was responsible for the cataleptic symptoms. The lesions were too varied in shape and extent. And there is the possibility to be considered that the effect was not produced by the destruction but rather

by the zone of irritation surrounding the lesion. The case is further complicated by the fact that other cats with similar lesions showed no signs of catalepsy. The symptom complex when obtained was, however, so characteristic and differed so markedly from anything which we have seen in a rather extensive experience with lesions in other parts of the cat's brain that we believe we are here dealing with a definite syndrome and that additional experiments will enable us to fix more definitely the location of the lesion, whether destructive or irritative, which is required to produce catalepsy in cats.

DISCUSSION. Lesions made by sticking a knife into the deeper parts of the brain are necessarily too extensive and too poorly localized to have much value in such a problem as this. The figures given by Spiegel and Inaba (1927) indicate that most of their lesions were unilateral and that they were not at all similar to those in our cataleptic cats. Reference should also be made to the experiments of Hess (1931) who was able to induce sleep in cats by electrical stimulation of deeply situated parts of the brain. But, since his figures indicate that this result was obtained from such widely separate and functionally diverse regions as the septum between the anterior horns of the two lateral ventricles, the head of the caudate nucleus, the anterior group of thalamic nuclei, the habenular trigone, the superior colliculus, and certain other points more ventrally situated in the lateral wall of the third ventricle, it is hard to see how such experiments could be interpreted as favoring the existence of any closely integrated mechanism regulating the change from the waking to the sleeping state.

There is a remarkable similarity between the behavior of our cataleptic cats and some patients with encephalitis lethargica in which an association of general muscular rigidity with a peculiar lethargy produces a clinical picture closely resembling catalepsy. The ptosis frequently seen in these patients indicates a lesion in the rostral part of the oculomotor nucleus. From pathological studies it is known that the lesions in this disease, while somewhat diffuse in their distribution, are most abundant in the region of transition between the midbrain and diencephalon. On the basis of a study of such cases von Economo (1930) postulated the existence of a center in the region between the mammillary bodies and the third nerve which is responsible for the pathological sleep exhibited by these cases. He conceives of this as a center from which inhibition spreads to the cortex. According to this conception the effective lesion would have to be an irritative one. One might with equal reason think of this as a region which when active radiated excitation to the cortex and that when this excitation was removed lethargy resulted (Pette, 1930). The experiments of Bard (1928) showing that cats, from which all of the brain rostral to the hypothalamus had been removed, were very irritable and exhibited sham rage, might be interpreted so as to lend some support to the latter

hypothesis. But in spite of the widespread interest in this subject during recent years due to epidemics of encephalitis lethargica, accurate information on the subject is far too meager to make such speculation profitable (Kleitman, 1929).

It is, however, generally recognized by clinical neurologists that pathologically prolonged sleep, which except for its duration resembles normal sleep and from which the patient can be readily aroused to clear consciousness only to drop off to sleep again as soon as he is left undisturbed, is a symptom which points to a lesion in the gray matter of the brain stem in the region of transition of the cerebral aqueduct into the third ventricle (Müller, 1931). In encephalitis lethargica the lesions are rather widely scattered through the basal ganglia and brainstem; and although the region of transition between mesencephalon and diencephalon seems to be the region where the lesions are most abundant such cases do not offer very satisfactory evidence as to the localization of the particular lesions responsible for the lethargy. A more satisfactory localization is offered by the case of Pette (1923). A vascular lesion of sudden onset resulted in paralysis of both oculomotor nerves and somnolence, which beginning on the day of onset, persisted until the death of the patient three months later. This sleep differed from coma in that the patient could be readily awakened to clear consciousness but would fall to sleep again as soon as he was left to himself. Autopsy showed an irregular but sharply circumscribed lesion in the tegmentum of the mesencephalon and floor of the third ventricle. In the bibliography are given references to a few of the more important papers which present evidence from clinical neurology to show that lesions located in this region frequently cause somnolence.

SUMMARY

Bilateral electrolytic lesions in the region between the mammillary body and third nerve in cats often lead to a condition of somnolence and exaggerated muscle tonus of a very plastic type. Cats with such lesions offer a striking resemblance to patients with encephalitis lethargica of the cataleptic type. They will maintain for many minutes unusual postures into which they have been placed by the experimenter and the ease with which they can be molded into statuesque postures seems to be directly related to their somnolence and the paucity of voluntary movements.

The lesions in these cats occupy the region of transition between mesencephalon and diencephalon which is known to be involved in certain types of pathologic sleep in man. Since the lesions observed in such cases have never been very sharply localized little is known about the nervous mechanisms involved. The possibility of producing prolonged sleep in cats by placing restricted bilateral lesions rostral to the third nerve offers an opportunity for determining more accurately the location of this mechanism and

for the study of the manner in which it regulates the change from the waking to the sleeping state. Such experiments may also lead to an explanation of why in some patients somnolence is associated with increased plastic tonus, a combination of symptoms known as catalepsy, and why in others it is associated with muscular relaxation.

BIBLIOGRAPHY

- BARD, P. 1928. *This Journal*, lxxxiv, 490.
- VON ECONOMO, C. 1930. *Journ. Nerv. and Ment. Dis.*, lxxi, 249.
- FULTON, J. F. AND P. BAILEY. 1929. *Journ. Nerv. and Ment. Dis.*, lxix, 1, 145, 261.
- HESS, W. R. 1931. *Compt. Rend. Soc. d. Biol.*, cvii, 1333.
- HIRSCH, E. 1927. *Monatschr. f. Psychiat. u. Neurol.*, lxiii, 113.
- INGRAM, W. R. AND S. W. RANSON. 1932. *Arch Neurol. and Psychiat.*, in press.
- KLEITMAN, N. 1929. *Physiol. Rev.*, ix, 624.
- MÜLLER, L. R. 1931. *Lebensnerven und Lebenstrieb*. Chapter by S. REGEL-S-
BERGER, 483, Springer, Berlin.
- PETTE, H. 1923. *Deutsch. Zeitschr. f. Nervenheilkunde*, lxxvi, 1.
1930. *Klin. Wochenschr.*, ix, 2329.
- PÖTZL, O. 1927. *Monatschr. f. Psychiat. u. Neurol.*, lxiv, 1.
- SPIEGEL, E. A. AND C. INABA. 1927. *Zeitschr. f. d. gesamt. exp. Med.*, lv, 164.

THE RATE OF FORMATION OF CEREBROSPINAL FLUID IN ETHERIZED CATS

LOUIS B. FLEXNER AND H. WINTERS

From the Department of Anatomy, Johns Hopkins University

Received for publication June 8, 1932

The literature contains no accurate data on the normal rate of formation of cerebrospinal fluid; as Levinson (1929) has said, this is still a matter of conjecture. Such reports as are at hand have come from observations on the rate of flow of the fluid from fractured portions of the skull and spinal column, or from measurement of the quantity escaping from a cannula placed in the subarachnoid space of an animal. In numerous cases of cerebrospinal rhinorrhea the amount of fluid flowing from the nose has been found to be from 96 cc. to 720 cc. per day (Levinson, 1929). Falkenheim and Naunyn (1887), using dogs and the method of cannulation of the subarachnoid space found a rate of fluid-escape varying from 36 to 240 cc. in 24 hours. Estimates of the normal rate of formation of cerebrospinal fluid from data of this kind have these objections: cerebrospinal fluid pressure is equal to the resistance of the artificial opening and so is far below normal, and no account can be taken of the amount of fluid being absorbed during the period of observation. The interpretation of data from cases of injury to the skull or vertebral column is faced with the additional complications which may come from trauma to the central nervous system and its coverings. These considerations led Weed (1922) to the opinion that estimates of the normal rate of fluid-formation available to him were all probably too high.

METHODS. It has been our aim to devise a method which permits measurement, under normal intraventricular pressure, of the amount of cerebrospinal fluid leaving the aqueduct of Sylvius, during the course of several hours. For this purpose it was necessary to establish and maintain a water-tight block at, or rostral to, the cerebellar peduncles so that fluid escaped neither into the subarachnoid space via the foramina of Luschka nor past the block directly into the region of the cisterna magna. It was further necessary to have an instrument which permitted measurement of changes in fluid-volume occurring at known pressures.

Our first efforts to obtain a water-tight block were made by attempting to catheterize the aqueduct with a small, silk ureteral catheter. This method has, in our hands, been entirely unsuccessful for even after suc-

cessful catheterization leaks were always present between the catheter and the walls of the aqueduct. Our next attempts were made with a small, soft rubber catheter of size 10. This was placed in the fourth ventricle against the opening of the aqueduct and held in position by cotton packs and agar placed rostral to the cerebellar peduncles. To prevent leakage around the catheter a ring of bone wax about 2 cm. high was fixed to the occiput and atlas, and mercury then poured into the ventricle and the bone wax cup. In this way it was possible to maintain a pressure of about 2 cm. of mercury on the cotton packing. This last method has yielded data which we feel to be valid but it has the disadvantages of being both laborious and uncertain.

Most of the data to be reported here have come from measurement of fluid flowing through a catheter placed against the aqueduct of Sylvius and surrounded by a balloon shaped to secure blockage of the ventricle. Glass models of the portion of the fourth ventricle rostral to the cerebellar peduncles were blown from paraffin-wax casts. Rubber balloons were then made over these glass models according to the method of Reynolds and Friedman (1930) which involves use of pure gum caoutchouc dissolved in carbon tetrachloride and vulcanization by sulphur chloride. A small, soft rubber catheter was then introduced into and through the balloon, and, for the purpose of dilating the balloon, a second small catheter was connected. The union of the catheters to the balloon was made by use of thin celloidin. After properly placing the balloon with its catheters in the rostral portion of the ventricle, blockage was accomplished by distending the balloon through the catheter which ended within it. If, then, the fit between balloon and ventricular wall were water-tight, all fluid which escaped from the aqueduct must have flowed from the catheter which penetrated the balloon.

In all these experiments, the amount of fluid leaving the catheter was measured with a bubble-manometer such as used by Weed, Flexner and Clark (1932). This consisted of a long tube of 1 mm. bore with a scale attached so that the distance traversed by a bubble of air in a column of Locke's solution could readily be ascertained. The volume of sections of the tube was measured with mercury and length could therefore be translated into volume. A movable fluid-reservoir of relatively large diameter (15 mm.), attached to one end of the manometer, permitted determination of the rate of cerebrospinal fluid-escape at any desired intraventricular pressure.

It was of course of first importance to be certain that no artifacts entered into the method. There were several possible sources of error. The experiments were made in a room in which no precautions were taken to insure a constant temperature. It was found, however, if care was taken to fill the catheter and its connections with the manometer completely with

Locke's solution and to rid them of all air, that the position of the bubble in the manometer was practically invariable with relatively extreme temperature changes. This factor appeared, therefore, to influence in no appreciable way the correctness of the observations.

More important than this was the demonstration of a water-tight block between the ventricle and the catheter. At the end of each experiment the animal was killed and the apparatus left in position. Drift of the bubble in the manometer toward the animal was then taken as evidence of leakage and the experiment discarded. The test was very sensitive and hence apparently reliable. It led to our discarding most of the results obtained with mercury-blockage and about two out of every three experiments in which balloons were used.

The balloons were distended either with air or water. When water was used, the pressure within the balloon amounted to about 160 mm. of water. It is evident that a small leak in the body of the balloon might have permitted escape of water from the balloon into the ventricle and so produced movement of the bubble in the manometer not due to escape of cerebrospinal fluid. This possibility could be investigated immediately post-mortem with the apparatus in place. In the presence of a leak, the bubble in the manometer instead of being stationary would have moved away from the dead animal. There was the unlikely possibility that a leak of this kind might have been approximately compensated for by a leak between the walls of the balloon and the ventricle and that, therefore, a stationary bubble post-mortem provided a somewhat uncertain test of the correctness of observations made during life. To rule out this slight possibility, some of the balloons were distended with air instead of water. Should air escape from the interior of the balloon into the ventricle and affect the position of the bubble, it must, during the course of an experiment, pass into the catheter and be observable in the glass portions of the apparatus. With the same artifact in mind, the ventricles of animals used in the experiments with mercury were examined post-mortem for any of the metal which might have passed the cotton and agar plug. We have had practically no difficulty from this source of error.

These experiments were all performed on adult cats. The animals were kept at normal body temperature under light surgical ether anesthesia throughout the period of observation. A mid-line incision was made through the skin over the occiput and back of the neck and the muscles attached to the occipital bone and atlas separated and retracted to give exposure of these bones and the occipito-atlantoid ligament. After exposing the cerebellum by rongeur-ing away a portion of the occipital bone, the dura and arachnoid over the cisterna magna were incised. The cerebellum was then carefully lifted away from the medulla with a spatula and the fourth ventricle entered. After removing the fluid in the ventricle with

cotton pledgets, the aqueduct could readily be seen. The balloon and its catheters were then placed in the rostral portion of the ventricle. Hemorrhage caused no difficulty. At the end of the experiments, all animals were promptly killed.

Throughout these experiments, observations were made with an intraventricular pressure of 110 mm. water \pm 10 mm., account having been taken of the resistance of the apparatus. This is a pressure about equal to the average normal as found by Weed and Hughson (1921b) for adult cats.

EXPERIMENTAL DATA. The data from the observations on etherized adult cats may best be presented under several topics:

TABLE 1

EXPERIMENT NUMBER	BODY WEIGHT	BRAIN WEIGHT	CHORIOID PLEXUS WEIGHT	METHOD OF BLOCKAGE	PERIOD OF OBSERVA- TION	RATE OF C.S.F. FORMATION
	grams	grams	mgm.		hours	cc. per day
CV-1				Mercury	2.0	13.4
CV-2				Mercury	2.0	13.2
CV-5				Balloon and H ₂ O	3.5	8.8
CV-6	2,600	18.0	40	Balloon and H ₂ O	6.5	15.9
CV-8	2,500	20.0	30	Balloon and H ₂ O	2.5	15.8
CV-11	3,200	25.6	32	Balloon and H ₂ O	4.0	10.1
CV-23	3,500	23.5	25	Balloon and H ₂ O	2.0	10.6
CV-28	2,900	22.0	25	Balloon and air	4.5	11.4
CV-30	3,500	22.5	15	Balloon and air	2.0	13.7
CV-31	3,500	23.0	35	Balloon and air	2.5	13.7
CV-32	3,300	25.6	30	Balloon and air	1.0	9.3
CV-34	3,000	25.0	40	Balloon and air	1.0	12.0
CV-35	2,500	20.5	25	Balloon and air	1.0	10.5
Average.....						12.1

Rate of flow of cerebrospinal fluid from the aqueduct of Sylvius. Table 1. presents in summary form observations on the rate of flow of cerebrospinal fluid from the aqueduct of Sylvius. On the basis of measurements taken over periods from one to six and one-half hours in 13 adult cats, it has been found that an average of 12.1 cc. of cerebrospinal fluid per day leave the aqueduct. Among individual animals, the quantity appeared to vary between 9 cc. and 16 cc. It is apparent from the table that this variation in rate was not to be correlated with differences of body weight or brain weight. In 10 of the experiments, the chorioid plexuses of lateral and third ventricles were removed, and weighed after surface fluid had been absorbed by filter paper. In all instances, an attempt was made to remove carefully the tela chorioidea from the plexus; nevertheless, a con-

siderable error undoubtedly came from this source. The variations in rate of fluid-escape found no explanation in these weights.

Striking differences in the character of the fluid-stream leaving the aqueduct were noted among the experiments and, on occasion, at different

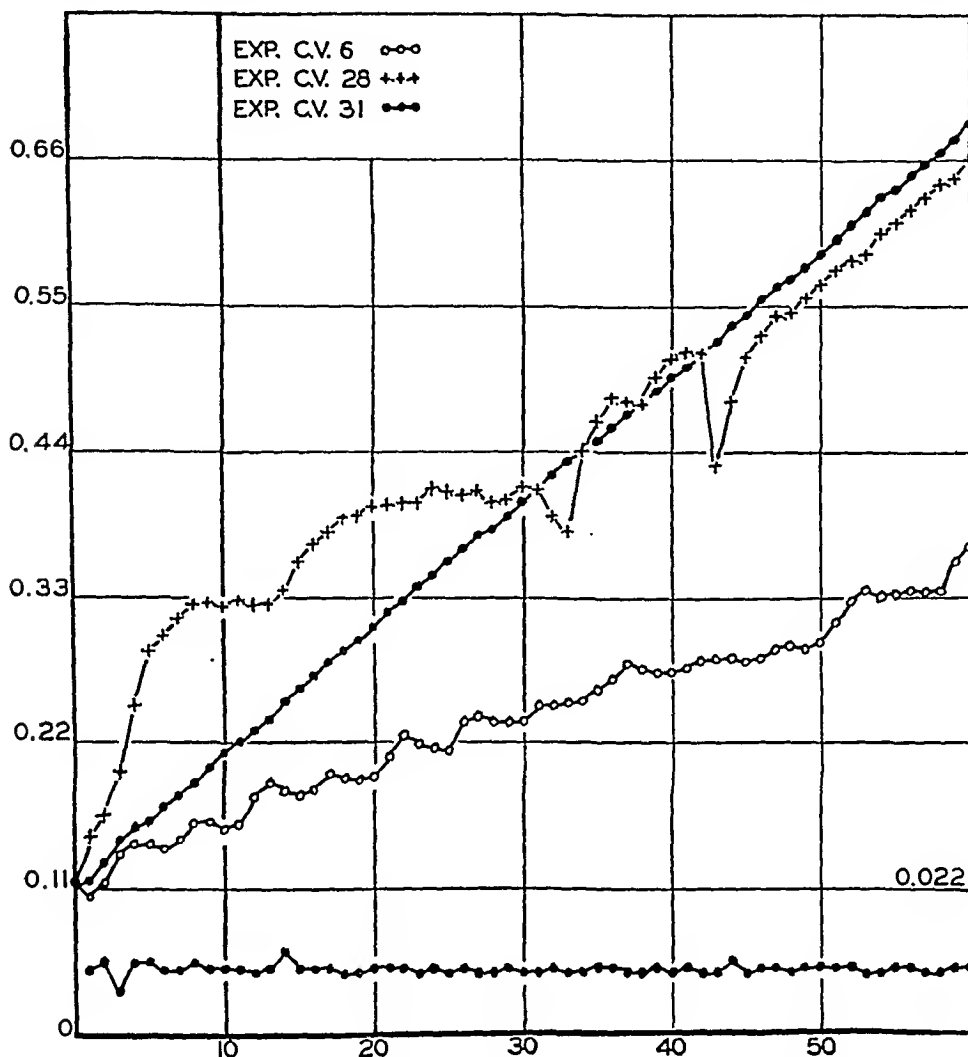


Chart 1 (cats, experiments CV-6, CV-28 and CV-31). The ordinates on the left represent volume in cubic centimeters of cerebrospinal fluid-flow from the aqueduct of Sylvius; the abscissae, time in minutes. The curves portray the three types of flow observed in a series of 13 cats. The ordinates on the right represent volume in cubic centimeters of cerebrospinal fluid-flow and evaluate the lowest curve which expresses rate of flow per minute for experiment CV-31.

periods of the same experiment. Some of the observations demonstrated a rate of flow which was extraordinarily constant, minute after minute, for several hours. In such experiments, exemplified in chart 1 by experi-

ment CV-31, the rate per minute as plotted showed little deviation from a horizontal straight line and the total quantity of fluid which had escaped at any particular time lay on a line of practically constant slope. In this experiment CV-31 almost all of the measurements showed a rate per minute varying only between 0.0088 and 0.0099 cc.

In other animals, exemplified in chart 1 by experiment CV-6, there were long periods in which fluid-flow from the aqueduct occurred in very regular cycles. Periods of active flow were followed by phases in which no fluid left the aqueduct; a rate of about 0.01 cc. per minute suddenly or gradually gave way to a period of two to four minutes in which flow ceased. The whole cycle in experiment CV-6 lasted for an average of about five minutes and the whole period of cyclic flow for about 80 minutes.

Finally, there was a group of experiments in which the rate of flow was highly irregular. As is shown by experiment CV-28 of chart 1, here again there were well defined cycles in which large rates of flow from the aqueduct were followed by periods of cessation. These cycles, however, were very irregular in their times of duration and in their forms; periods of unusually active flow amounting to as much as 0.06 cc. per minute alternated with periods of cessation or intervals during which fluid from the manometer was sucked back into the ventricles. It is to be noted in the graph of this experiment that the period of extreme irregularity was followed by a period in which rate of flow was almost as constant as in experiment CV-31.

Several of the animals showed irregularities of a different sort. Chart 2 shows two such experiments, CV-6 and CV-11, in which the form of the curve and the amount of fluid-escape varied greatly from hour to hour. In CV-6, which showed the most extreme variations, maximum fluid-escape for an hour amounted to 1.5 cc., and minimum, to 0.3 cc. In experiment CV-5 of chart 2, on the other hand, although the curve of fluid-flow was irregular, the amounts of fluid produced per hour were almost identical and showed only slightly greater variation than experiment CV-31 of chart 1. Four of the 13 experiments showed irregular rates from hour to hour and were of much the same type as CV-6 and CV-11; six presented only slight variations in hourly flow though three of these were of the irregular form of experiment CV-5; in three, observations were made for only an hour.

Evidence of ventricular volume changes. Intracranial arterial and venous pressures were recorded simultaneously with rate of cerebrospinal fluid-escape in several of the experiments. Measurements of the pressure in the circle of Willis presented little difficulty; this was accomplished by cannulation of the peripheral end of the carotid artery. Intracranial venous pressure was less readily determined. The superior sagittal sinus in most cats is too narrow to permit puncture with a needle as suggested for dogs by Weed and Hughson (1921a). To measure pressures from

the torcular herophili is often difficult and the method has certain dangers of inaccuracy.

Venous pressures in these experiments have been recorded from the peripheral end of the external jugular vein. This vein in the cat and dog is the largest vein of the neck, the internal jugular being, in all but rare

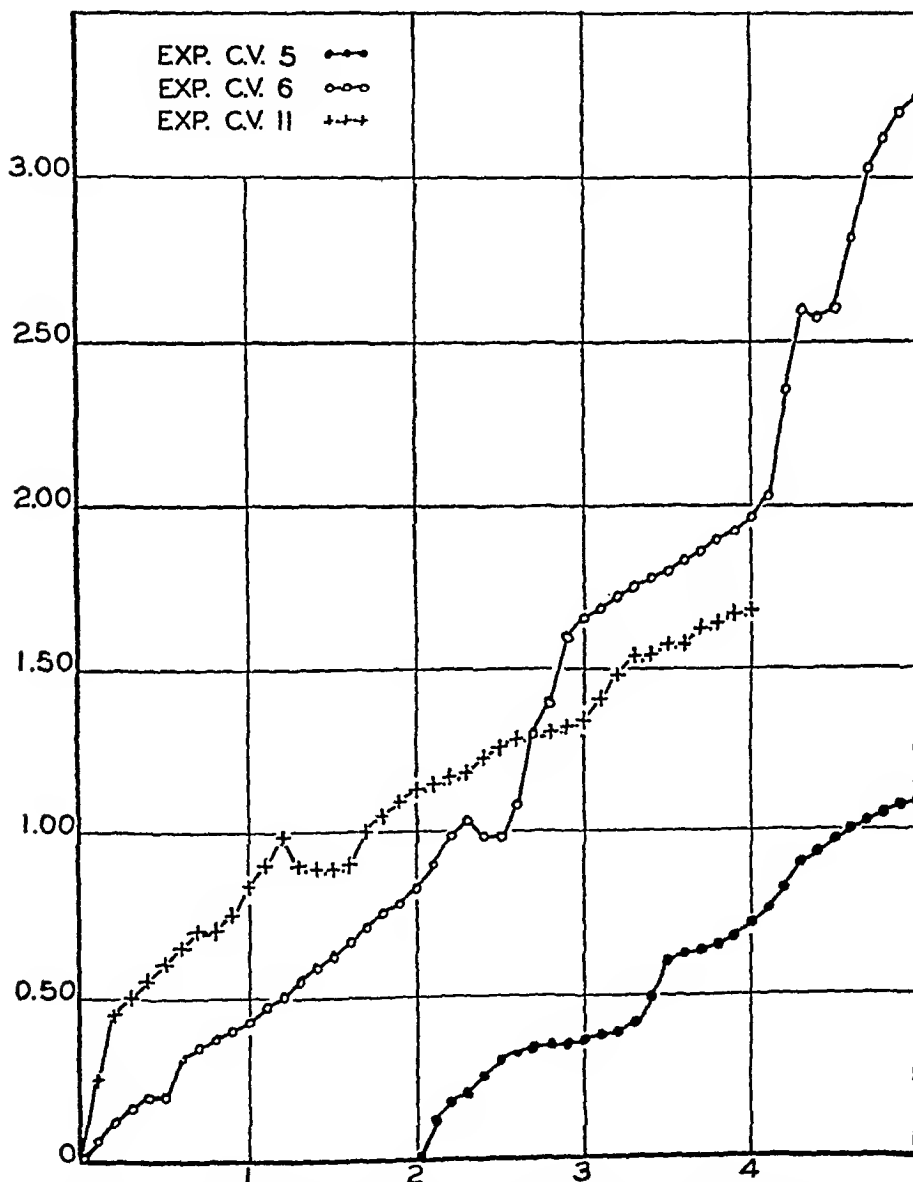


Chart 2 (cats, experiments CV-5, CV-6 and CV-11). The ordinates represent volume in cubic centimeters of cerebrospinal fluid-flow from the aqueduct of Sylvius; the abscissae, time in hours. The curves portray the irregularities of rate of flow over long periods observed in certain experiments (CV-6 and CV-11); and the constancy found in others (CV-5).

instances, of a caliber too small to allow cannulation. Several dissections of the external jugular veins of cats and dogs were made and the relations of these vessels in both animals found to be practically equivalent. In all instances the vein in the neck was found to receive a large branch from within the skull. It was consequently argued that the peripheral end of the vein might be used to record intracranial venous pressure.

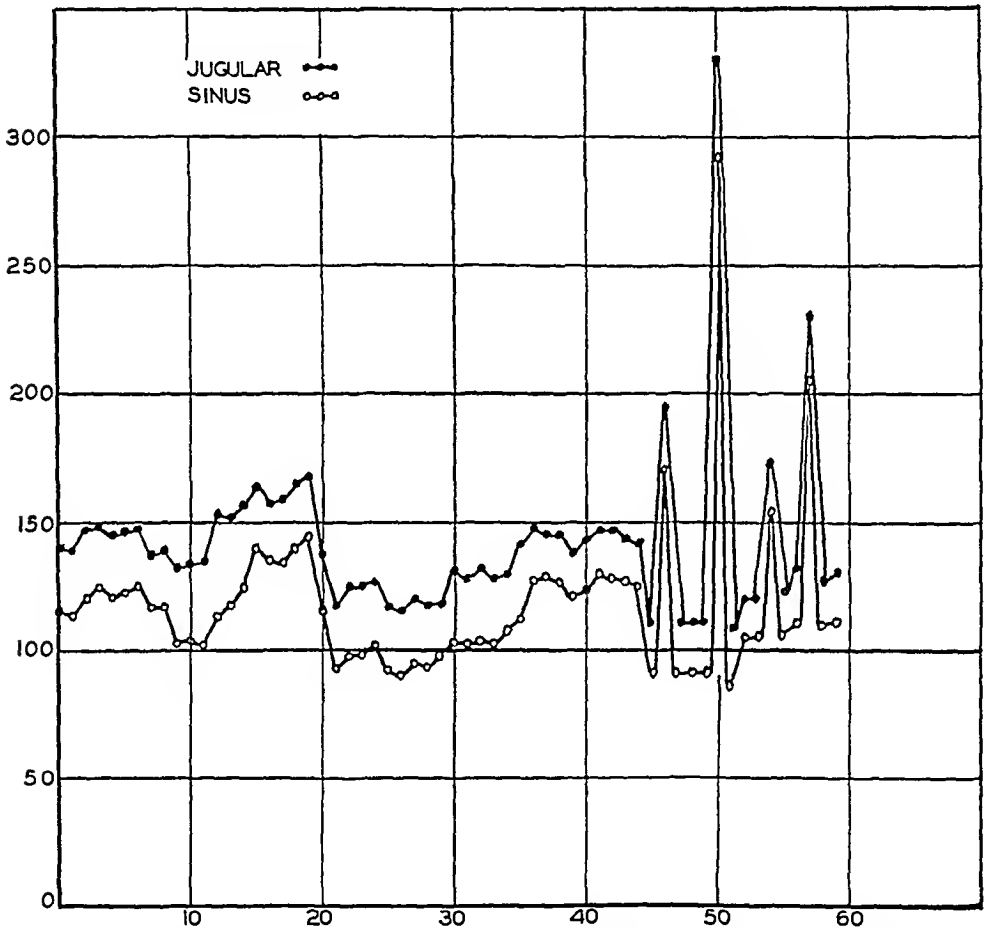


Chart 3 (dog, experiment CV-18). The ordinates represent pressure in millimeters of normal saline solution; the abscissae, time in minutes. The curves show the equality of pressure changes which occurred in the superior sagittal sinus and the peripheral end of an external jugular vein. In the interval from 45 to 65 minutes, intracranial venous pressure was altered by pressing on the intact external jugular vein. Other changes occurred spontaneously.

To put this possibility to test, simultaneous pressure readings were taken from a needle placed in the superior sagittal sinus of a dog according to the method of Weed and Hughson, and from a cannula in the peripheral end of the external jugular vein. Chart 3 presents some of the data from

such an experiment. It is seen that spontaneous pressure changes occurred simultaneously and to almost the same degree in both vessels. Moreover, pressure on the intact external jugular vein produced small or large pressure changes of the same magnitude in the two vessels. The same sort of result followed decrease or increase of intracranial pressure consequent to withdrawal of cerebrospinal fluid from the subarachnoid space or injection of the fluid into it. The pressure in the external jugular vein was always found to be slightly higher than in the superior sagittal sinus; in experiment CV-18 of chart 3 this difference was between 20 and 25 mm. of normal saline, the maximum difference found in three experiments. These observations appeared, consequently, to justify use of the peripheral end of the external jugular vein in the cat and dog for measurement of changes in intracranial venous pressure.

Because of the likelihood of altering the blood supply to structures of the ventricles by cannulation of carotid artery and external jugular vein, arterial and venous pressures were measured in only three of the experiments reported here. Experiment CV-34 of chart 4 presents typically the results of such observations.

It was first necessary to determine the effects on intracranial blood pressures of the necessary manipulations in and about the fourth ventricle. At the time marked by arrow L of the graph (chart 4), the dura and arachnoid over the cisterna magna were incised with escape of cerebrospinal fluid and fall of its pressure to the level of the atmosphere. This procedure was without effect on arterial pressure, but, as was anticipated, caused a decrease in venous pressure amounting in different cases to from 10 to 30 mm. of normal saline solution.

At the time marked by arrow B of the graph (chart 4), the balloon and its catheters were placed in the ventricle and the balloon then distended. In two of the three experiments conducted in this way, distention of the balloon was accompanied by an increase of intracranial arterial pressure amounting to about 10 mm. of mercury. Venous pressure, however, never showed a measurable change. These findings coupled with the finding post-mortem of a fourth ventricle only slightly dilated, led to the conclusion that the pressures used in distending the balloon introduced no important error into the observations.

Changes in intracranial blood volume apparently led to variations in the volume of the ventricles. The bubble of the manometer, with the balloon in place and distended, invariably showed pulsations just as does the fluid meniscus of a manometer connected to the subarachnoid space. Arterial pulsations equivalent to a ventricular volume change of approximately 0.001 cc. and respiratory pulsations amounting to a volume change of approximately 0.01 cc. were frequently noted. These changes were of course of great regularity and influenced in no way the rate of flow per min-

ute from the aqueduct. They demonstrate, however, that distension of the arteries following systole or emptying of the veins with inspiration produce changes in ventricular volume.

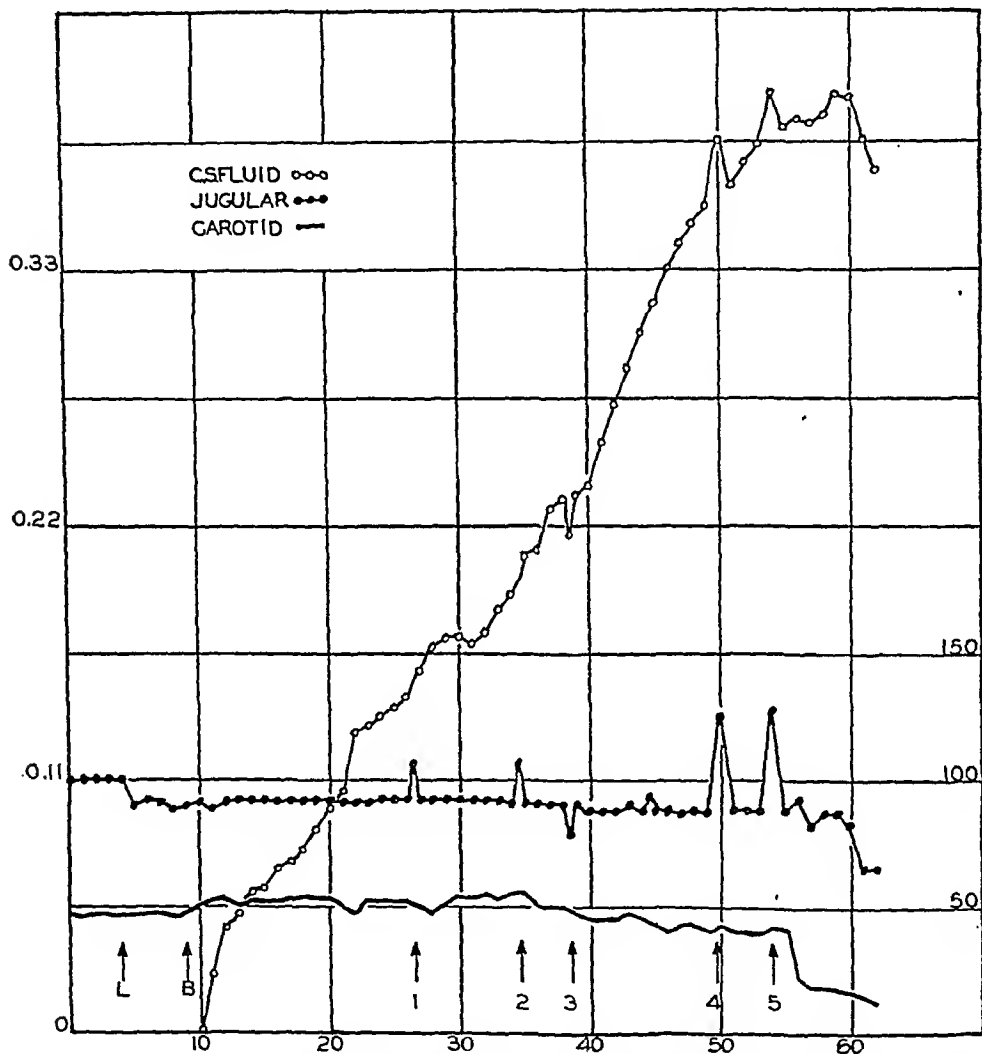


Chart 4 (cat, experiment CV-34). The abscissae represent time in minutes. The ordinates on the left represent volume in cubic centimeters of cerebrospinal fluid-flow from the aqueduct of Sylvius; the ordinates on the right, pressure in millimeters of normal saline solution or mercury (carotid pressure). The curves show the relations found between changes in rate of cerebrospinal fluid-flow and changes in intracranial blood pressures. The significance of the arrows is given in the text.

Changes of the same general sort, not related, in the same way, however, to heart beat or respiration were frequently observed during the course of the experiments. Experiment CV-6 of chart 1, for example, showed

periods as long as three minutes in which fluid was aspirated from the manometer into the ventricle. In other experiments such as CV-28 of chart 1 the change was more striking and as much as 0.1 cc. of fluid was sucked into the ventricle in the course of one minute. On the other hand it has been noted that as much as 0.3 cc. of fluid may, within about 30 seconds, be suddenly expelled from the ventricles. Very often the original position of the bubble was quickly reestablished but at times there was no recession.

Simultaneous measurement of intracranial arterial and venous pressures and of cerebrospinal fluid-flow from the aqueduct have furnished data which may offer an adequate explanation of these observations. Experiment CV-34 of chart 4 indicated that the changes in arterial pressure likely to occur in the course of a well conducted experiment, with the animal in good condition, did not alter in an appreciable way the rate of flow. Small increases or small decreases in the pressure within the circle of Willis produced no apparent change in rate of cerebrospinal fluid-escape. Rather marked changes in venous pressure occurring without apparent cause or on occasion as a result of movement of the animal were, however, often correlated with changes in the rate of flow. Thus the transitory increases in venous pressure marked by arrows 1 and 2 in chart 4 were accompanied by transitory increases in the rate. Increase in venous pressure from pressure on the intact external jugular vein also caused a momentary increase in flow (arrows 4 and 5 of chart 4). A decrease in venous pressure as marked by arrow 3 caused a decrease in flow. Reestablishment of venous pressure was accompanied by a resumption of the original rate of flow. It is of interest to note that with approach of the animal's death, fluid in the manometer was sucked into the ventricles coincident with sharp falls in arterial and venous pressures. Obviously, however, marked changes in rate of flow did occur without evidence of change in intracranial vascular pressures. This lack of correlation, very frequently observed, deserves the same emphasis as does the correlation.

DISCUSSION. Three methods, fundamentally alike but differing in important details, have been shown to yield results of the same magnitude for the rate of flow of cerebrospinal fluid from the aqueduct of Sylvius of etherized adult cats. Each experiment was tested as severely as was found possible for artifact and in the event of its demonstration, the experiment was discarded. Two efforts were made to test further the measurements obtained from these methods by plugging the aqueduct with cotton and placing a needle through the corpus callosum and into the third ventricle. These attempts failed because of leakage around the needle.

An average of 12.1 cc. per day of cerebrospinal fluid has been found to flow from the aqueduct of the adult cat under ether anesthesia. Whether or not this is to be taken as an accurate statement of the rate of formation

in the normal cat is at the moment uncertain. Analysis of this possibility awaits investigation of the effects of ether on the rate of formation of the fluid.

It would of course be of much additional value if an average could be given for the amount of fluid which leaves the four ventricles to flow into the subarachnoid space. Technical difficulties have made direct measurement of this quantity impossible. It has been found, however, that the chorioid plexus of the fourth ventricle weighs on the average about 25 per cent as much as the plexuses of the other three ventricles. If the assumption be made, and this may well be entirely unjustifiable, that the amount of fluid formed from a single plexus of any individual animal is approximately a direct function of the plexus weight, then an addition of 3 cc. per day must be made. This would give an average outflow of cerebrospinal fluid from the ventricles of 15 cc. per day.

The average value given here has come from experiments lasting from one to six and one-half hours, and averaging a little more than two and one-half hours in duration. It can be urged that these intervals are too short to permit deductions as to the amount of cerebrospinal fluid formed over a period of 24 hours. Rate of formation of cerebrospinal fluid may well show large fluctuations in the same individual as has been suggested by Hart (1927) and has been demonstrated in some of the experiments reported here. A considerable error may consequently appear to have been introduced in the calculations for a day. The only answer which can be made to this criticism is that a rather large series of animals have been studied and that they have all given results of the same magnitude. It is likely that they presented various degrees of activity of fluid-formation and that, therefore, the average figure presented affords a fair estimate of the rate per day in the cat.

It has been pointed out that there are apparently three distinct modes of flow, varying from almost perfect regularity through regular cycles of changing flow to a type of great irregularity. No particular type is to be taken as characteristic of an individual animal. It appears probable that in all animals, over a long period of time, the character of the fluid-stream leaving the aqueduct changes in these three possible ways.

Such changes as these must find their explanation in two causes: first, actual variations in the rate of formation of cerebrospinal fluid and second, variations in the volume of the ventricles. That changes in ventricular volume do occur as the result of variations in the blood volume of their walls, appears to be substantiated by considerable evidence. Throughout these experiments, rise in venous pressure has been taken as an index of increased distention of the veins and hence an increase in venous blood volume; and fall in venous pressure, as evidence of decrease in venous blood volume. It has been shown that a decrease in venous pressure was ac-

accompanied by aspiration of small amounts of fluid into the ventricles and that increased venous pressure apparently caused expulsion of fluid from the ventricles. These changes, as far as they have been studied, have been characterized by small reaction times, the maximum noted being five minutes, most occurring in less than a minute. The ventricles, therefore, apparently are a part of the extensive mechanism of reciprocal compensation within the bony coverings of the central venous system. They share in the reactions which keep total volume of cerebrospinal fluid, blood and central nervous system practically constant as is demanded by the Monroe-Kellie doctrine.¹

There are, however, changes in rate of flow which are not accompanied by measured changes in blood pressure. These are frequently different from those just mentioned in that they last for long periods of time and appear due to actual variations in the rate of formation of cerebrospinal fluid. An explanation for these variations is entirely problematical. To argue that they are independent of arterial pressure is misleading for the pressure as measured in the circle of Willis is but a crude indication of pressure-changes taking place within the capillaries of the chorioid plexus; and knowledge of this quantity can alone provide an unequivocal analysis. In the same way, measurement of venous pressure gives only a view of changes occurring within the large sinuses of the head and tells little of small but probably important changes in the venules and veins of the structures of the ventricles.

Care has been taken throughout this report to speak of rate of flow or escape of cerebrospinal fluid, rather than its rate of formation. This has been done because the description of results was largely concerned with changes taking place over short periods of time. These changes, as has been discussed, appear related not only to actual rate of formation but to ventricular volume-changes as well. In evaluating measurements made over a long period, however, it appears wholly justifiable to speak of rate of formation of cerebrospinal fluid. Ventricular volume changes, over these periods, become inconsequential.

It is of interest to inquire how nearly the amount of cerebrospinal fluid formed within the ventricles approaches the total quantity produced by the animal. An analysis of this problem involves a quantitative knowledge of the amounts of fluid escaping from the perivascular spaces and from the blood vessels of the subarachnoid space. Exact information in this regard is lacking and any statement in consequence is conjectural. The work of Weed (1922) and Schaltenbrand and Bailey (1928), however, indicates that normally the perivascular spaces contribute a very small quantity of fluid to the subarachnoid space. Nor is there any good evidence that

¹ For a general discussion of reciprocal compensation, see L. B. Flexner, J. H. Clark, and L. H. Weed, *This Journal*, 1932, ci, 292.

cerebrospinal fluid leaves the vessels of the subarachnoid space. With our present knowledge, therefore, it is perhaps justifiable to conclude that measurement of the amount of cerebrospinal fluid leaving the ventricles gives a quite accurate estimate of the total quantity of fluid produced. This conclusion is only tentative, however, and with further investigation, may well prove to be untenable.

SUMMARY

Using three methods, fundamentally alike but differing in important details, the average rate of flow of cerebrospinal fluid from the aqueduct of Sylvius in the etherized adult cat has been found to be 12 cc. per day. The rate in a series of 13 cats varied between about 9 cc. and 16 cc. of fluid per day. These differences found no explanation in weight of body, brain or chorioid plexuses.

Three distinct types of flow were found among the animals. Changes in pressure within the circle of Willis afforded no explanation of these findings. Some of the deviations from a constant rate of flow, however, were correlated with intracranial venous pressure-changes and are considered to be evidences of ventricular volume change.

BIBLIOGRAPHY

- FALKENHEIM, H. AND B. NAUNYN. 1887. *Arch. f. exper. Path. u. Pharm.*, xii, 261.
HART, D. 1927. *Arch. Surg.*, xv, 943.
LEVINSON, A. 1929. *The cerebrospinal fluid*. 3rd ed., St. Louis.
REYNOLDS, S. R. M. AND M. H. FRIEDMAN. 1930. *This Journal*, xciv, 696.
SCHALTENBRAND, G. AND P. BAILEY. 1928. *Journ. f. Psychol. u. Neurol.*, xxxv, 199.
WEED, L. H. 1922. *Physiol. Reviews*, ii, 171.
WEED, L. H. AND W. HUGHSON. 1921a. *This Journal*, lviii, 101.
1921b. *This Journal*, lviii, 53.
WEED, L. H., L. B. FLEXNER, AND J. H. CLARK. 1932. *This Journal*, c, 246.

EFFECT OF POSTERIOR PITUITARY EXTRACTS ON THE CONSTITUENTS OF THE BLOOD

H. E. HIMWICH, F. W. HAYNES AND J. F. FAZIKAS

From the Department of Physiology, School of Medicine, Yale University, New Haven, Connecticut

Received for publication June 10, 1932

The effects of the posterior pituitary extract on blood constituents have been extensively studied in recent years. The rise in blood sugar obtained with pituitrin (Partos and Klatz-Klein, 1921; Burn, 1928; Clark, 1925; Tingle and Imrie, 1926) is effected also by pitocin and pitressin (Geiling, 1932; Nitzescu and Benetato, 1930; Bacq and Dworkin, 1930). Other experiments (Himwich and Fazikas, 1930; Bischoff and Long, 1931) have shown that pitressin and pituitrin also cause a rise in blood lactic acid. Since it has been found that the respiratory metabolism is affected differently by the three pituitary extracts (Himwich and Haynes, 1931) a differential study of their effects on the lactic acid and glucose content of the blood of unanesthetized and amytalized animals has been made in an attempt to explain the various changes produced in metabolic rate. The concentration of the blood as a criterion of blood dilution was measured since an increase in blood volume after pituitrin has been observed (Konschegg and Shuster, 1915; Underhill and Pack, 1923).

METHOD. Chloretone-free pituitrin, pitressin, and pitocin were injected subcutaneously into amytalized and unanesthetized dogs in doses of 1.0 to 4.6 pressor or oxytocic units¹ per kilo. These doses were not lethal and the dogs were in good condition at the end of the experiment. Injections were made every 15 or 30 minutes and blood samples of 5 to 15 cc. were drawn from the femoral artery, usually at intervals of two hours. Analyses were made for lactic acid by the method of Friedemann, Cotonio, and Shaffer (1927) and for sugar by the method of Hagedorn and Jensen (1923). Total solids were determined from the dry weight of 1 cc. of plasma. In order to determine the effect on the plasma concentration of merely drawing blood control samples were taken from unanesthetized animals. Because of the irregularity of the results under amytal anesthesia, 2 dogs were decerebrated before study.

¹ One cubic centimeter pituitrin contains 10 oxytocic and 10 pressor units. One cubic centimeter pitocin contains 10 oxytocic units and 1 cc. pitressin contains 10 pressor units. These substances were generously supplied by Parke, Davis & Company.

RESULTS. The results of this series of experiments are summarized in table 1. A plus indicates a rise, a zero no change, and a minus a fall in the blood constituent. The figures show the number of experiments in which such changes occurred. Changes greater than the experimental error (at the foot of each column) are considered significant.

It may be seen that pitressin and pitocin as well as pituitrin raised the blood glucose in unanesthetized animals. The average increase in milligrams per cent was 57 for pituitrin, 29 for pitressin and 17 for pitocin. The rise in sugar was less consistent in the case of amytalized dogs and was even reversed after pitocin and pitressin. Chloralose, however, did

TABLE 1
Changes in blood constituents after pituitary extracts

	BLOOD GLUCOSE			LACTIC ACID			TOTAL SOLIDS OF PLASMA		
	+	0	-	+	0	-	+	0	-
Pituitrin unanesthetized.....	3	0	0	2	2	0	0	0	6
Pituitrin amytalized....	3	0	1	0	2	1	0	0	1
Pitocin unanesthetized.....	3	0	0	0	5	1	0	1	5
Pitocin amytalized....	0	0	3	0	1	0	2	0	2
Pitressin unanesthetized.....	4	0	0	5	0	0	1	0	4
Pitressin amytalized....	1	0	3	2	1	0	2	0	4
Control unanesthetized.....							0	0	4
Experimental error....	±2.5 mgm. %			±2.5 mgm. %			±3 mgm. cc.		
Remarks.....							In 2 of the controls the fall was preceded by a slight rise		

not have the same effect as amytal since injections of pitressin produced a great rise in the concentration of blood sugar in each of 3 observations.

Determinations of lactic acid in unanesthetized dogs reveal that pitressin caused a rise of 9 to 27 mgm. per cent whereas pitocin had little effect. A few experiments in which tissue lactates were determined have failed to show definite results.

The change in total solids after pitressin and pitocin indicated a dilution of the blood of unanesthetized animals similar to the dilution found after pituitrin. The effect may be partly due to merely drawing blood.

Experiments on decerebrate dogs are difficult to evaluate since after an initial rise blood glucose and lactic acid decrease (Himwich, Koskoff and Nahum, 1930). In spite of this fact pitressin caused a rise in lactic acid in both experiments on decerebrate animals.

DISCUSSION. Himwich and Haynes (1930) found that pitressin decreased oxygen consumption of unanesthetized rats. Recently Geiling (1932) noted that when pitressin was administered the venous blood returned in an arterialized condition indicating that the tissues with the exception of the brain were removing little oxygen from the blood. As a result of diminished oxidations it might be expected that the liver glycogen would break down to glucose and so appear in the blood and that muscle glycogen would undergo cleavage to lactic acid which would also be found in the blood. This would appear to be the case as a rise in the lactic acid of the blood plasma is caused by pitressin but not by pitocin which does not depress the oxygen consumption. Pituitrin lowers the metabolic rate to some extent and probably increases the blood lactic acid.

Experiments with posterior pituitary extract in general may throw some light on the action of pitressin, its pressor fraction. A diminution of liver glycogen after pituitary extract has been found (Burn and Ling, 1929), suggesting a greater output of glucose or a decreased formation of glycogen from lactic acid. Other workers (Lawrence and McCance, 1931; Bischoff and Long, 1931) have failed to confirm these results since they could find no definite change in muscle or liver glycogen after pituitrin. The latter observers also suggest an increased removal of sugar by the peripheral tissues which makes explanation even more difficult.

Raab (1926) has demonstrated that the effect of pituitrin on blood fat is mediated by centers in the diencephalon. It is known that "Dial" acts on the hypothalamus and medulla (Fulton, Liddell and Rioch, 1930) and it is probable that amytal decreases the sensitivity of these centers on which pituitrin may act. In a similar manner avertin anesthesia arrests the action of pituitrin injected into the brain ventricles (Cushing, 1931). The dosage which is effective in unanesthetized dogs may be without effect on animals under amytal. Thus it is seen in the present experiments that with all three extracts the blood sugar and lactic acid often remained unchanged or were lowered in anesthetized animals. We cannot say, however, that the only action of posterior pituitary extract is its direct action on the hypothalamus (in which lie the chief centers of the autonomic nervous system) since pituitrin and pitressin also decrease the oxygen intake of excised tissues (Himwich, Finkelstein and Humphreys, 1931) which are removed from nervous control.

SUMMARY

Observations of the effects of pitressin, pitocin and pituitrin on plasma glucose, lactic acid and total solids were made on 17 amytalized, 21 unanesthetized and 2 decerebrate dogs.

Pitressin, pitocin and pituitrin increase the blood glucose in unanesthetized animals but often lower it in amytalized animals.

Pitressin increases plasma lactic acid in unanesthetized dogs whereas pitocin has little effect.

Pitressin and pitocin probably cause a dilution of the blood similar to that observed after pituitrin.

We wish to thank Miss Marjorie H. Hurlburt for aid in preparation of the manuscript.

The expenses of the research were met in part by a grant-in-aid from the Research Fund of the Yale University School of Medicine.

We wish to thank Parke, Davis & Co. and Dr. Oliver Kamm for a generous supply of the posterior pituitary extracts.

BIBLIOGRAPHY

- BACQ, Z. M. AND S. DWORKIN. 1930. *This Journal*, xcv, 605.
BISCHOFF, F. AND M. L. LONG. 1931. *This Journal*, xcvii, 215; xcix, 253.
BURN, J. H. 1928. *Quart. Journ. Pharm.*, i, 509.
BURN, J. H. AND H. W. LING. 1929. *Quart. Journ. Pharm.*, ii, 1.
CLARK, J. A. 1925. *Journ. Physiol.*, lix, 466.
CUSHING, H. 1931. *Proc. Nat. Acad. Sciences*, xvii, 248.
FRIEDEMANN, T. E., M. COTONIO AND P. A. SHAFFER. 1927. *Journ. Biol. Chem.*, lxxiii, 335.
FULTON, J. F., E. G. T. LIDDELL AND D. McK. RIOCH. 1930. *Journ. Pharm. Exper. Therap.*, xl, 423.
GEILING, E. M. K. 1932. *Johns Hopkins Hosp. Bull.*, li, no. 1.
HAGEDORN, H. E. AND B. N. JENSEN. 1923. *Biochem. Zeitschr.*, cxxxv, 46.
HIMWICH, H. E. AND J. FAZIKAS. 1930. *Proc. Soc. Exp. Biol. and Med.*, xxviii, 331.
HIMWICH, H. E., R. FINKELSTEIN AND K. E. HUMPHREYS. 1931. *Proc. Soc. Exp. Biol. and Med.*, xxix, 233.
HIMWICH, H. E. AND F. W. HAYNES. 1931. *This Journal*, xcvi, 640.
HIMWICH, H. E., Y. D. KOSKOFF AND L. H. NAHUM. 1930. *Journ. Biol. Chem.*, lxxxv, 571.
VON KONSCHIEGG, A. AND E. SHUSTER. 1915. *Deutsch. Med. Wochenschr.*, li, 1091.
LAWRENCE, R. D. AND R. A. McCANCE. 1931. *Biochem. Journ.*, xxv, 570.
NITZESCU, I. AND G. BENETATO. 1930. *Compt. rend. Soc. Biol.*, ciii, 1359.
PARTOS, A. AND F. KLATZ-KLEIN. 1921. *Zeitschr. f. d. gesamt. Exp. Med.*, xxv, 99.
RAAB, W. 1926. *Zeitschr. f. d. gesamt. exper. Med.*, xlix, 179.
TINGLE, C. D. AND C. B. IMRIE. 1926. *Journ. Physiol.*, lxii, 2.
UNDERHILL, F. P. AND G. T. PACK. 1923. *This Journal*, lxi, 520.

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